EFFECTS OF LAND COVER AND RIPARIAN BUFFERS ON COLD-WATER FISH ASSEMBLAGES IN UPPER SOUTH FORK NEW RIVER HEADWATER STREAMS

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by
CRISTINA LEE SANDERS

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CRISTINA LEE SANDERS
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APPROVED BY:

Dr. Shea R. Tuberty
Chairperson, Thesis Committee

Dr. Jeff D. Colby
Member, Thesis Committee

Mr. Jason H. Selong
Member, Thesis Committee

Dr. Zack E. Murrell
Chairperson, Department of Biology

Dr. Mike McKenzie
Dean, Cratis D. Williams School of Graduate Studies

Abstract

EFFECTS OF LAND COVER AND RIPARIAN BUFFERS ON COLD-WATER FISH ASSEMBLAGES IN UPPER SOUTH FORK NEW RIVER HEADWATER STREAMS

Cristina Lee Sanders
B.S., University of Phoenix
M.S., Appalachian State University

Chairperson: Dr. Shea R. Tuberty

Climate change combined with expanding urbanization and changes to land-use pose a serious threat to many cold-water species as temperature continues to increase. Riparian vegetation is an essential component of a stream ecosystem that reduces runoff, improves bank stability, and provides shading that regulates the water temperature thresholds. Western North Carolina has many sensitive cold-water fish species that are vital to the stability of the ecosystem and also provide substantial revenue to the state including brook (*Salvelinus fontinalis*), brown *(Salmo trutta*), and rainbow trout (*Oncorhynchus mykiss*).

This study focused on the effects of biogeophysical components on cold-water fish assemblages within the eight headwater sub-basins that comprise the Upper South Fork New River watershed and vary widely in ability to support sensitive and endemic fish species. The relative abundance of cold-water fish were sampled by electrofishing 16 sites during the spring and summer of 2019 and then compared to GIS determined riparian corridor density and height measurements, the percentage of sub-basin wide impervious surfaces, Bank Erosion Hazard Index (BEHI) scores, Wolman Pebble Count scores, water temperature, and

specific conductivity. Fish size and weight were also recorded to determine size-class composition and condition metrics.

The monthly maximum stream temperature did surpass static laboratory determined thermal suitability limits for brook and brown trout; and diurnal fluctuating laboratory determined limits for rainbow trout (20.0-22.5 °C) in 14 sites. However, the regression analyses of riparian heights ($R^2 = 0.05$) and densities ($R^2 = 0.07$) within each sampling reach did not significantly reduce stream temperature between sites. Furthermore, weight and length measurements for cold-water species was consistent between sites when temperature stress was expected to cause metabolic effects. Conductivity concentrations ranged from 18.5-501.2 µS/cm between sub-basins and were highly reduced as the percentage of impervious surfaces decreased. Although water chemistry did not impact cold-water fish conditions in the USFNR; stream bank erosion, benthic substrate, riparian height, and impervious surfaces did. The relative abundances of cold-water fish increased with quality of habitat which emphasizes the importance for management decisions to continue to preserve or improve habitat conditions based on these findings.

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A special thanks to my lab mate Grant Buckner for his help with both field work and intellectually stimulating problem-solving conversations. Most importantly this project would not have been possible without the help of my rock-solid undergraduate volunteers Hannah Woodburn, Ashley Joyner, Jasper Yoke, and Kim Beamer. I cannot begin to thank you all enough.

Lastly, I would like to acknowledge the Department of Biology at Appalachian State University for providing financial support and the IACUC committee for approving this research.

Dedication

I would like to dedicate this thesis to my husband Brian for always supporting me and keeping me sane during the times I felt overwhelmed and for helping me with field work when I needed an extra set of hands. I would also like to dedicate my research to my 8-year old son Landon for always brightening my day and being the brightest young aquatic ecologist that ensured all of the fish were returned to the streams gently and unharmed.

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Foreword

This thesis has been formatted in accordance with the styling requirements for submission to the peer-reviewed journal *Freshwater Biology*.

Introduction

Riparian zones are an essential part of the stream ecosystem, especially during the summer months when shading can block solar radiation and combat rising water temperatures (Cross *et al.*, 2013; Poole & Berman, 2001). The largest influence in stream temperature changes occurs through air-water energy exchanges (Dugdale *et al.*, 2017). Solar radiation is the largest provider of this heat exchange, especially during the hot, summer months. Since the sun has the greatest influence on water temperature, riparian vegetation can act as a buffer to limit the shortwave solar radiation, however, the shading ability of riparian vegetation depends on the size of the stream (Imholt *et al.*, 2011). A 2001 study by Poole & Berman listed riparian shade as highly important for thermal buffering in stream orders 1 & 2 and moderately important for stream orders 3 & 4, with minimal influence on larger streams. Riparian shading is not the only important characteristic in headwaters, Poole & Berman 2001 also list phreatic ground water as highly important in stream orders 1 & 2 and moderate in stream orders 3 & 4. Increasing urbanization and changes in land-use has resulted in a significant reduction of these vegetative zones within the United States and five of the warmest years on record since 1880 have all occurred since 2015 with 2019 listed as the second warmest year (Rutherford *et al.*, 2004; NOAA, 2020). Therefore the combination of both climate change and urbanization impacts poses a threat to cold-water sensitive aquatic species.

Increasing temperatures can have a detrimental effect on many cold-water fish species of the southern Appalachian Mountains that are particularly vulnerable to specific temperature thresholds. Among these species is the brook trout (*Salvelinus fontinalis*), which is the only trout species native to North Carolina. Other cold-water species in the southern

Appalachian region include the introduced brown trout (*Salmo trutta*) andrainbow trout (*Oncorhynchus mykiss*), mottled sculpin (*Cottus bairdi*) and the cool-water blacknose dace (*Rhinichthys atratulus*). Salmonids, which include trout species, have been extensively documented as being sensitive to increasing temperatures. The known thermal tolerances between salmonid species does differ depending on the type of study conducted. A study by Carlson *et al.*, 2017 found the following preferred growth temperature ranges: brown trout (12.0-20.0°C), brook trout (11.0-20.5°C), and rainbow trout (12.0-22.5°C). The stress from increasing temperature can affect physiological and behavioral responses. Directly, changes in metabolism can hinder growth rate and overall survival rates (Bell, 2006; Carlson *et al.*, 2017; Wehrly *et al.*, 2007). Reproduction can also be impacted by temperature differences because spawning ques for salmonids are based on a specific temperature range, prolonged elevated temperatures can affect spawn timing which possibly leads to the eggs hatching during an undesirable period with increased competition or limited resources (Bell, 2006; Cook *et al.*, 2018; Merriam *et al.*, 2017). Indirect effects caused by an increase in water temperature can alter behavioral interactions by making the fish change normal movement patterns. Moving to a previously unoccupied section of the stream can lead to abnormal competition between other species, differences in habitat selection, and dietary differences due to changes in prey availability or suitability (Bell, 2006; Wehrly *et al.*, 2007).

In addition to reducing solar inputs, riparian zones can also stabilize stream banks and reduce the amount of contaminant runoff that enters a waterway. Ionic concentrations, such as chloride from road salt and sulfates, increases in rivers and streams as natural lands are removed and replaced by impervious surfaces (Morgan *et al.*, 2012; Hedrick *et al.*, 2010). Chloride from road salt readily washes into the waterways and elevates concentrations in the winter months but can also accumulate within the soil and groundwater which can increase levels during the warmer months as well (Morgan *et al.*, 2012). Most studies focus on temperature; the effects of environmentally relevant concentrations of freshwater salinization on salmonids and other cold-water fish species is not well documented, but it is a growing area of concern (Hintz & Relyea 2017). In parts of western North Carolina, chloride inputs from road salt applications continue to accumulate in the susceptible low-order headwater streams. A study by Hintz & Relyea 2017, found that rainbow trout exposed to environmentally relevant concentrations of NaCl did reduce fish growth but had low effects on mortality over a 25-day period. This could occur because salinity has an influence on food intake and metabolic rates (Boeuf & Payan, 2001). Freshwater salinization can also negatively impact lower trophic levels. Sensitive aquatic macroinvertebrates, which are the primary food source for many fish species in western North Carolina, react negatively to increasing levels of road salt (Fleetwood, 2017). Predicted climate changes, coupled with reduced riparian zones, could not only affect biodiversity but also harm the substantial income that the fishing industry profits. Mountain trout fishing brings in substantial revenue and job opportunities for the state. In 2014, the annual revenue was approximately $210.7 million from trip expenses, fishing equipment, and licensing (NCWRC, 2015). Revenue aside, the mountain trout fishing industry alone created 3,200 jobs in North Carolina (NCWRC, 2015).

The Upper South Fork New River (USFNR) is a unique community that contains both natural and urban headwater streams that supports wild trout reproduction in western North Carolina despite heavy development within the watershed. The productivity of naturalized trout populations in a continuously growing environment has amazed fisheries biologists throughout the region. The presence of federally owned forested headwaters running off the Blue Ridge Parkway's high elevation peaks as well as urban areas with high percentages of impervious surfaces vastly changes the biogeophysical components between sub-basins. This rare combination of forested and urbanized streams warranted further research to determine which environmental parameters are driving local fish assemblages as well as identify which environmental variables need to be preserved to deter future impacts as human development continues to increase.

In this study, cold-water fish assemblages were collected within the USFNR watershed using electrofishing techniques in the spring and summer months to determine seasonal stressors. The fish were captured from a total of 16 sites, 2 per sub-basin, were identified to the species level and weight and length measurements were recorded. A 150-meter transect was used for the sampling area and 3 separate mesohabitats (riffle, run, pool, bank) were sampled at every site. The data from the captured fish was compared to a multitude of associated environmental factors to determine which variables influence cold-water fish assemblages in small, headwater streams. Water chemistry data was collected from Eureka Manta 2 in-situ water chemistry sondes that recorded a data point every 15 minutes at the catchment in each of the 8 headwater sub-basins that comprise the USFNR by the AppAqua research cluster at Appalachian State University (ASU, 2020). Stream sedimentation was analyzed using a Bank Erosion Hazard Index (BEHI) and a modified Wolman Pebble Count at each of the 16 sampling transects (WVDEP, 2019a; WVDEP, 2019b). Riparian vegetation was calculated using GIS to determine the height and percent of vegetative density within a 25-foot corridor width along each sampling transect. Lastly, land cover data was calculated using 6-inch aerial photography to determine the percentage of impervious surfaces within each sub-basin in the USFNR watershed upstream of the water chemistry data sondes.

The first objective of this study was to assess cold-water fish assemblages within the USFNR headwater streams and relate the assemblages to water temperature, conductivity, sedimentation, stream-bank erosion, and riparian heights/densities to determine which environmental variables have an impact on cold-water species. The second objective was the development of riparian height and density models and impervious surface data within the USFNR using Geographic Information Systems (GIS). This study is aimed to provide data to assist Local, State and Federal agencies in the development of strategies to maintain biodiversity and recreational mountain fisheries through informed policy making, smart growth, as well as conservation efforts, monitoring, and habitat restoration.

Materials and Methods

Study Sites

The USFNR watershed is nestled within the southern Appalachian Mountain range in Boone, North Carolina. It is divided into eight sub-basins: State Farm, East Fork, Goshen, Middle Fork, Boone Creek, Hodges Creek, Winkler Creek, and Flannery Fork (Figure 1) (Kinlaw, 2019). This watershed contains headwater stream sub-basins that are influenced by a wide spectrum of either impervious surface and heavily forested areas. Therefore, stream conductivities within each sub-basin vastly differ and have been categorized from low, moderate, to high based on long-term historical data that has been collected by the AppAqua research cluster at Appalachian State University since 2010. The heavily forested sub-basins with low conductivity (Goshen Creek, Winkler Creek, and Flannery Fork) serve as reference streams to compare to the somewhat impacted streams (East Fork and Middle Fork) and severely impacted streams (Boone Creek and Hodges Creek) with high impervious surface percentages.

Study Fish

Within the USFNR watershed, sixteen 150-meter transects were sampled for cold-water fish assemblages in Spring (May and early June) and again in warmest period of summer (late July and August) to represent the temporal differences that can impact cold-water fish distribution. Two transects were sampled per sub-basin of the USFNR watershed (Table 1). Within each of these transects four mesohabitats (e.g. riffle, run, pool, streambank) were identified and sampled using a three-pass depletion method to ensure every species present within the transect was recorded. Every mesohabitat was sampled for 100 seconds to give a total of 1200 seconds per entire 150-meter transect using a Smith Root LR-24 backpack electrofisher (Holcomb *et al.*, 2013). This electro-fisher automatically calculates the direct current (DC) pulse output using the anode and cathode to measure the conductivity of the stream (Smith Root, 2020). This technique ensured that all captured species were returned unharmed (ASU IACUC Protocol 18-17;5/1/2018).

The focal species for this study were brook trout (*Salvelinus fontinalis*), brown trout *(Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), mottled sculpin (*Cottus bairdii*), and blacknose dace (*Rhinichthys atratulus*) because these are the primary species that occupy the low diversity, high elevation, cold-water trout streams of the southern Appalachian mountain region (NCDENR, 2013). The relative species abundance percentage of the focal species was calculated using the following formula:

$$Relative\ Species\ Abundance\ \% = \frac{N\ Focal\ Species}{N\ Total\ Species}\ x\ 100 \qquad (1)$$

N = number of collected fish

This calculation was used to control for the variability of stream orders between sub-basins and to include the coinciding non-focal fishes. The relative abundance calculations were appropriate because the fish collection protocol was standardized for every site which provided comparable samples.

The focal fish data was then compared to water chemistry, GIS riparian height and density calculations, GIS impervious surface percentages, pebble count scores, and BEHI scores using a joint-plot Principle Component's Analysis (PCA) in PC-ORD v.7 to identify influential variables. The variables of interest revealed by the PCA were then compared using linear regressions to test for relationships and the significance of each regression was determined in SigmaPlot V14.0 using either a Pearson Correlation or Spearman-Rank Correlation depending on the results of the Shapiro-Wilk test of normality.

Additionally, each captured fish was identified to genus and species, weighed, and measured for total length to determine size class composition metrics; this data was also recorded for the annual collection report under the NC Wildlife Resources Commission Scientific Collection Permit # 19-SFC00038. The Fulton's condition factor (K) was calculated for summer collected young of year (YOY) and adult brown trout using the following formula (Froese, 2006):

$$K = 100\frac{W}{L^3} \qquad (2)$$

K = Fulton's condition factor; W = whole body wet weight (g); L = total length (cm)

Fulton’s condition factor is used to account for normal fish growth based on ‘cube law’ because fish do not proportionately grow in length and weight, instead fish increase more in weight than length (Froese, 2006). Fulton’s condition factor was only calculated for brown trout due to their abundance and widespread distribution throughout the watershed; whereas the total number of other captured trout species was sparse and insufficient to compare among sub-basins.

Bank Erosion Hazard Index (BEHI)

The stream bank was assessed to determine the potential for bank erosion and stability using the Bank Erosion Hazard Index (BEHI). In this method the root depth to bank height ratio, percentage of root density, streambank angle, and percentage of vegetative surface protection were calculated, and the scores were factored into an overall BEHI index value as described below in Table 2 (Harman & Jones, 2017). The BEHI index value categorized each stream as very low, low, moderate, high, very high, or extreme; with very low being the least eroded (Harman & Jones, 2017). These measurements were taken 5 times (~once in 100 linear ft) per 150-meter transect when both sides of the stream bank were homologous in nature (Harman & Jones, 2017). If the opposing banks were not homologous, a measurement was taken for both sides. The total index values were combined and averaged to represent the overall BEHI for each 150-meter sampling transect.

Modified Wolman Pebble Count

A coarse benthic assessment was surveyed using a modified Wolman pebble count to determine the quality of the stream bed at each sampling site. Every 150-meter sampling transect was divided into 16 sub-transects (~30 feet apart) where 25 particles were randomly selected using the zig-zag method from bank to bank. The particle located at the tip of the right toe was selected using the tip of the index finger to avoid sampling bias. The intermediate axis of each particle was measured using a gravelometer and recorded (USFWS, 2004). The particles were identified into one of the following size categories: sand < 2 mm, gravel 3-64 mm, cobble 65-255 mm, and boulder > 255 mm (WVDEP, 2019a). The particles were then compared to a point value and given a score; the total score categorized the stream benthos as poor (≤ 2.5), marginal (≤ 3), or good (> 3) (WVDEP, 2019). Additionally, the data was organized by coarse particle size frequency and cumulative frequency distributions in Microsoft Excel 2010 using a modification of the methods recommended by Wolman (1954). An additional measure was employed to determine the median particle class size (D_{50}) of the collected particles when distributions were arranged from smallest to largest. This allowed the determination of a single size class value for spatial and temporal comparison of particle sizes.

Water Chemistry

Water chemistry data was recorded using in-situ Eureka Manta 20+ water quality sondes in Boone Creek, East Fork, Flannery Fork, Goshen Creek, Middle Fork, and State Farm that remain in the stream at all times. Winkler's Creek and Hodge's Creek were recorded with Eureka Trimeter sondes. The sonde locations within the streams are listed in Table 3. The Eureka Manta 20+ water chemistry sondes record temperature, pH, specific conductivity, dissolved oxygen, and depth every 15 minutes. The Eureka Trimeter sensors record temperature, specific conductivity, and depth every 15 minutes. The sensors were calibrated and serviced monthly by the ASU AppAqua research cluster to ensure accuracy. During these intervals, the recorded data was uploaded to a field laptop and checked for inconsistencies that can occur due to dead batteries, fouled probes following high water events, or failing probes. After the quality assurance check, the data was ready to be uploaded to the Graphical User Interface (GUI) on the Department of Geography and Planning.

The water temperature and conductivity data was analyzed using a Kruskal-Wallis one-way Analysis of Variance on ranks combined with the Dunn's method post-hoc test to determine statistical differences between sites and sampling seasons.

LiDAR-Geiger Riparian Calculations

The width of each riparian corridor was created using ArcMap 10.5 and the percentage of riparian density within each corridor was calculated using 2018 Geiger-LiDAR point cloud data. Based on the percentage of riparian density, each riparian corridor was classified as either poor, fair, or good condition in accordance with the Catchment Assessment of the North Carolina Stream Quantification Tool (Harman & Jones, 2017). Riparian zones classified as poor condition have less than 50% of the stream length containing greater than 25 feet of riparian corridor width, fair condition has greater than 25 feet of corridor width in 50-80% of the stream length, and good condition has greater than 25 feet of riparian vegetation in more than 80% of the stream reach (Harman & Jones, 2017).

The classification of riparian density and height were analyzed using 2018 QL1 aerial Geiger-LiDAR data that was obtained from the N.C. Emergency Management's Spatial Data

Download website (NCDPS, 2018). The downloaded Geiger-LiDAR data was collected at 8 points per meter and is separated into the following relevant class codes: 0-never classified, 1-unassigned, 2-ground, 3-low vegetation, 4-medium vegetation, 5-high vegetation (NCDPS, 2018). The individual tiles that correspond to each of the 16 collection sites in the USFNR were downloaded as individual .LAS files, imported into ArcMap, and converted into an .LASD dataset by creating a *NEW LAS Dataset* in the ArcCatalog. At this time the statistics were calculated to find the average point count of the data and the georeferencing system was verified. Two LAS dataset layers were created, one for vegetation and one for bare-earth using the *Make .LAS Dataset Layer* tool and selecting the appropriate class codes. The vegetation layer used class codes 1, 3, 4, and 5 to include all vegetation and the unassigned values that may contain vegetation data; the bare-earth layer used only class code 2-ground data (ESRI, 2019). The newly created LAS datasets were then each converted to a raster using the *LAS Dataset to Raster* tool; the sampling value was set to 4 times the average point spacing value which was previously calculated during the statistics step (ESRI, 2019). Lastly, the *Hillshade* tool was used to provide a 3D representation of the layers (Figure 2). The 2018 6-inch aerial photography for Watauga County, NC was also imported into ArcMap 10.5 to compare to the bare-earth layer to verify the edge of the stream banks when drawing the sampling transects.

A total of 32 polygon transects were created to capture the riparian corridors at each of the 16 sampling sites (2 polygons per site to represent both stream banks). The corridor width was extended 25-feet perpendicularly from the edge of the stream bank for the entirety of the 150-meter sampling area using heads up digitizing. Initially a new shapefile feature class of multipoints was created in ArcCatalog using the same georeferencing system as the

LiDAR-Geiger data for every riparian corridor. Each corridor was drawn using “create features” in the *Editor Toolbar.* A point was created every 10-meters along the sampling area; this distance was selected so that the corridor would follow the curvature of a stream without cutting out essential vegetation data. From each multipoint, an additional multipoint was extended perpendicularly 7.62-meters (25-feet) from the stream bank to provide an equal distance throughout the transect.

A new shapefile for polygons was then created in ArcCatalog for each of the 32 riparian corridors and edited using the *Editor Toolbar* to connect the previously drawn multipoints and form a closed polygon (Figure 3). Each polygon was then converted into a raster using the *Feature to Raster* tool and the output cell size was set to 0.67 meters (4 times average point spacing) to keep the values consistent with the other layers (Figure 4). The canopy density was then calculated with the *.LAS Point Statistics as Raster* tool using the previously created vegetation raster with class codes 1,3,4, and 5 selected (ESRI, 2019). The *Is Null* and *Con* tools were used to convert all cells with no data to 0 to eliminate noise (ESRI, 2019) (Figure 5). These steps were repeated using the bare-earth raster with class code 2 selected (Figure 5).

After the vegetation and bare-earth layers were conditioned separately, they were added together using the *Plus* geoprocessing tool to produce the canopy density map (Figure 6) (ESRI, 2019). The combined layer was then converted to a floating point and divided to create a ratio from 0.0-1.0 which represents the canopy density; 0 = no vegetation and 1 = dense canopy (ESRI, 2019). To determine the percentage of riparian density within each of the sampling transects the data within each polygon raster was converted into a binary format by masking the raster using *Raster Analysis* and then reclassifying the data using the

Reclassify tool. The binary data was classified as 1 = vegetation and 0 = no vegetation or no data. The count data for each classification was located in the attribute table. To determine the percentage of riparian density the following formula was used:

$$\%Riparian\ Density = \frac{Vegetation\ Count\ (1)}{Vegetation\ Count\ (1) + Non - Vegetation\ Count\ (0)} \times 100\ (3)$$

The riparian height was also calculated in ArcMap 10.5 using the *Minus* tool by subtracting the LiDAR bare-earth layer from the LiDAR vegetation layer (Figure 7) (ESRI, 2019). The calculated height differences were then conditioned using the condition feature in the *Raster Calculator* to remove any negative values (Figure 7) (ESRI, 2019). The height within each sampling transect was determined by first using *Raster Analysis* to select the desired polygon transect raster and creating a mask. The sum, minimum, maximum, and mean canopy heights were calculated using *Zonal Statistics*.

Percent Impervious Surface Classification

Land cover data was classified as impervious or non-impervious surface using 2018 6-inch aerial photography for Watauga County, NC. The aerial photography was uploaded into ArcMap 10.5 and the delineated shapefile of the USFNR watershed boundary was extracted using the “Extract by Mask” tool in ArcToolbox. The imagery was then resampled using “Resample” to change the output cell size to 3.28 ft (1m) based on the work by Coffey 2011 to use with the Feature Analyst extension. The new feature class representing impervious surfaces was creating using heads-up digitizing by drawing polygons of 50 random impervious surfaces (parking lots, buildings, roads, homes). The 50 identified impervious surfaces were then used in conjunction with the “Supervised Learning” tool in Feature Analyst to create an algorithm that automatically detects impervious surfaces within the entire watershed. The Bull’s Eye 2 pattern was selected based on previous work by Coffey 2011 for its effectiveness at identifying impervious surfaces. The resulting shapefile from Feature Analyst identified the majority of impervious surfaces (Figure 8).

The impervious surface layer produced by Feature Analyst was far from perfect and the features needed to be manually edited to ensure accuracy. A fishnet grid was placed over the map and each grid was checked systematically to correct any missed or misidentified features (Figure 9). Once satisfied with each grid the color was changed to green and the next grid was corrected (Carlyle, 2013). Following corrections, the impervious surface polygons were converted to a raster using “Polygon to Raster” and were put into a binary format (0 = impervious; 1 = non-impervious). The percentage of impervious surface area for each individual sub-basin was calculated by masking the sub-basin of interest and using the following formula:

$$Impervious\ \% = \frac{Impervious\ (0)}{Impervious\ (0) + Non\text{-}Impervious\ (1)}\ x\ 100 \tag{4}$$

Results

Fish Collections and Condition Index

In the USFNR watershed fish from the following species were collected during sampling: brook trout (*Salvelinus fontinalis*), brown trout *(Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), mottled sculpin (*Cottus bairdii*), blacknose dace (*Rhinichthys atratulus*), creek chub

(*Semotilus atromaculatus*.), bluehead & bigmouth chub (*Necomis* spp.), fantail darter (*Etheostoma flabellare*), central stoneroller (*Campostoma anomalum*), northern hogsucker (*Hypentelium nigricans*), rosyside dace (*Clinostomus funduloides*), white sucker (*Catostomus commersonii*), longnose dace (*Rhinichthys cataractae*), redbreast sunfish (*Lepomis 15uratus*), green sunfish (*Lepomis cyanellus*), bluegill sunfish (*Lepomis macrochirus*), smallmouth bass (*Micropterus dolomieu*), silver shiner (*Notropis photogenis*), bluntnose minnow (*Pimephales notatus*), greenside darter (*Etheostoma blennioides*), Kanawha darter (*Etheostoma kanawhae*), New River shiner (*Notropis scabriceps*), rock bass (*Ambloplites rupestris*), and warpaint shiner (*Luxilus coccogenis*). In the spring, a total of 1,543 fish were collected, the summer total was 1,894 fish, and 3,437 were collected all together; the relative percentage of focal species and functional feeding groups (NCDENR, 2013) are described below in Table 4.

The majority of the captured trout were wild, naturalized fish with only a few being stocked as indicated by dull coloration and worn fins. Only the data from the focal species: brook trout, brown trout, rainbow trout, mottled sculpin, and blacknose dace was used for this study due to these species being listed as the primary occupants of cold-water trout streams in the southern Appalachian region (NCDENR, 2013).

To determine conditions for separate age classes amongst all sites, the Fulton Condition Factor was calculated and plotted against total length measurements for YOY and adult brown trout to identify sites with fish of noticeably reduced conditions among age classes of fish (Figure 10). Brook trout and rainbow trout were excluded from this analysis due to the limited number of individuals collected and sites with these species. Blacknose dace and mottled sculpin were also excluded due to low YOY data due to not making weight on the field scale. The brown trout YOY and adults were mostly within similar conditions at every site, with the exception of a few adult brown trout from Winkler, Goshen, and East Fork. Overall, the results of the individual fish weight and length regressions and the plotted Fulton Condition Factors revealed similar conditions for assemblages of brown trout throughout the USFNR watershed, and even during times of predicted thermal stress, indicating no relationship between sub-basins water quality and fish condition.

The total length and weight measurements that were recorded during the fish collection process were used to create fish condition indices for brown trout, blacknose dace, and mottled sculpin during the summer which is a critical time for fish to store energy for the winter and when thermal stress was expected to be at its greatest. Brook trout and rainbow trout were excluded due to their absence from multiple sites and low collection numbers (brook trout: 2 sites, 18 total collected; rainbow trout: 5 sites, 21 total collected). The length and weight measurements were log transformed to normalize the data. A linear regression of the three species was tested individually for the summer sampling season using SigmaPlot v14.0 to reveal any relationships during a period of predicted thermal stress. Fish weights above 400g (2 brown trout) were excluded from these calculations due to the field scales inability to record a weight that exceeded 400g; fish with weights of 0.0g (23 blacknose dace and 34 mottled sculpin) were also excluded due to inaccurately reporting the data for small fish with weights that ranged between 0.0-0.1g.

The condition regressions for each summer tested species was significant, with strong R^2 values indicating that the fish were similar in weight-length measurements across all sampling sites regardless of habitat conditions and water chemistry (Figure 11). The points that fell on the lower end of the weight at length spectrum were checked and verified to be from different sites for each of the focal species ensuring that the fish were in similar condition regardless of the impact level of any of the streams (Figure 11). The Spearman Ranks Correlation was performed on brown trout (*Salmo trutta*), blacknose dace (*Rhinichthys atratulus*), and mottled sculpin (*Cottus bairdi*) of all age classes due to failing the Sharpiro-Wilkes normality test. A positive correlation coefficient with a P-value of <0.001 resulted from every correlation test; indicating that fish weight and length tend to increase similarly at all sites regardless of habitat or water quality.

Principle Components Analysis (PCA)

A two-dimensional, joint-plot Principle Components Analysis (PCA) was created in PC-ORD to graphically represent the multivariate data used in this study and establish potential patterns in the data collected during the spring and summer (Figure 12). The relative abundance of each individual cold-water species and combined cold-water species abundance was compared to all measured environmental variables (water quality, instream habitat, riparian density and height, impervious surface percent, and BEHI). The interpretation of these PCA biplots is dependent on the vector angles: acute angles indicate a direct relationship between variables, orthogonal angles indicate no relationship, and obtuse vector angles indicate an inverse relationship between variables (Borcard *et al.*, 2018).

Based on the Eigenvalues, the first 2 principle components explain 71.21% of variation in the data for the spring collection and 63.9% of variation in summer collection data (Figure 12). Riparian height, pebble count, conductivity, impervious surface percent, and BEHI are the environmental variables that indicate potential relationships cold-water fish species and each other with one another in the USFNR in the spring, with addition of temperature in the summer results. The comparison of spring collected total relative cold-water species abundance to relevant environmental variables all showed an inverse relationship to conductivity, BEHI scores, and percentages of impervious surface but were directly related to riparian heights and pebble count scores which revealed that the relative abundances of cold-water fish were most impacted by the variables that contribute to habitat degradation. The relationships in the summer collection were similar to the spring results showing inverse relationships with conductivity, BEHI scores, and percentages of impervious surface with the addition of temperature and were directly related to riparian heights and pebble count scores (Figure 12). The inverse relationship with temperature in only the summer collection showed that more cold-water fish assembled in sites with colder water temperatures during the summer months when the water temperatures were greater compared to the spring.

The percent of impervious surfaces and BEHI scores showed a direct relationship to conductivity based on the acute angle of the vectors; this was expected because an increased percentage of impervious surface contributes to higher concentrations of conductivity combined with greater bank erosion that results from less dense riparian zones that do not adequately buffer contaminant runoff. Riparian height and pebble count scores also showed a direct relationship to each other which revealed that increased riparian height is improving the quality of the stream habitat by reducing the amount of fine substrate (Figure 12). The pebble count scores and riparian heights showed an inverse relationship compared to impervious surface percent, BEHI scores, and conductivity as indicated by the obtuse vector angles which suggested that better habitat with taller riparian zones and reduced sedimentation were opposed to degraded habitat with greater runoff potential (Figure 12).

Overall, the levels of conductivity were adequate representations of all-around habitat degradation.

In the summer collection, the Eigenvalues for the principle component 1 and 2 explain 63.93% of the variation within the dataset. The same environmental variables from the spring are also revealed during this sampling season, with the addition of temperature (Figure 12). Similar trends outlined in the spring collection are also true for the summer. The percent of impervious surfaces and BEHI scores are showing a direct relationship to conductivity; riparian height and pebble count scores are also showing a direct relationship to each other. Pebble count scores and riparian heights are showing an inverse relationship when compared to impervious surface percent, BEHI scores, and conductivity. The main difference between the seasonal collections is the addition of temperature being revealed as an influential variable in the summer when the threat of thermal stress on cold-water fish was predicted to be greater. In the summer, temperature is showing an inverse relationship with riparian heights and pebble count scores and direct relationships with impervious surface percentages, BEHI scores, and conductivity. The relative abundances of cold-water fish are reacting to riparian heights, pebble count scores, impervious surface percentages, and BEHI scores in the same manner as the spring, however; an inverse relationship is revealed for cold-water fish and temperature in the summer.

Modified Wolman Pebble Count & BEHI Scores

In the USFNR, the Modified Wolman Pebble Count scores ranged from good to marginal (Table 4). All the reference sites scored good; the moderately and highly impacted site scores ranged from good to marginal. The variance between pebble count scores within the USFNR was analyzed using the Kruskal-Wallis one-way ANOVA on Ranks; the median values between Wolman Pebble Count scores in the 8 sub-basins were not statistically different (P = 0.081). Additionally, the D_{50} scores were calculated using the cumulative sediment distribution percentage to determine the particle size that 50% of the samples at each site are equal to or smaller than (Table 5).

The calculated D_{50} scores were compared to cold-water fish species and salmonid species in both spring and summer fish collections to determine any significant relationships. Since the particle sizes were categorized as sand (< 2 mm), gravel (3-64 mm), cobble (65-255 m, and boulder (> 255 mm) instead of being measured individually, the D_{50} scores were numerically categorized according to Table 4. This conversion was done to make the scores usable for statistical analysis. In the spring collection the regression analyses of the substrate category were not significant for cold-water species (R^2=0.200, P=0.082). The summer relationship was statistically significant (P = 0.048), but the relationship was not strong based on the R^2=0.251 which reduces the ability to confidently describe D_{50} scores as an influential variable on the focal species within the USFNR watershed. However, the comparison of relative cold-water fish abundances to pebble count scores was significant for the spring (P = 0.002) and summer (P = <0.001) with moderately strong R^2 values (spring R^2 = 0.515; summer R^2 = 0.554) (Figure 13) that indicate that the percentage of cold-water fish within a sampling transect increases with pebble count scores, where the instream habitat contains a lower amount of fine particles.

The survey of the USFNR showed BEHI scores within the range low to high. All of the reference sites scored in the good category, the intermediate sub-basins ranged from good to moderate, and the poor sub-basins ranged from moderate to high erosion hazard levels

(Figure 14). The variance between BEHI scores within the USFNR was analyzed using the Kruskal-Wallis one-way ANOVA on Ranks; the median values between BEHI scores in the 8 sub-basins were not statistically different ($P = 0.077$) at this time. Although the difference was not statistically significant ($P > 0.05$), it was close and future policies need to maintain or improve bank stability conditions to prevent greater levels of erosions impact in the future.

Relative cold-water fish abundances were compared to BEHI scores using linear regression and this relationship was statistically significant for the spring and summer, both with P-values of 0.034 and R^2 values of 0.282. The strength of this inverse relationship shows abundances of cold-water species increasing with lower BEHI scores; indicating that cold-water fish prefer stream habitat with less bank erosion. Although the R^2 values for the BEHI scores are not very strong, significance makes sense given the relationship between cold-water fish and pebble count scores because bank erosion directly influences the amount of fine substrate in a stream. To ensure the pebble count and BEHI scores were interacting, a regression analysis of the two was performed. This comparison revealed that BEHI and pebble count scores had a negative relationship ($R^2 = 0.64$, $P = <0.001$) that indicated as the pebble count score decreases the BEHI score increases, which is expected since bank erosion directly contributes to stream sedimentation (Figure 15). This significant regression also reinforces the inverse relationship that was revealed by the PCA analysis.

Water Chemistry

The median monthly water temperature during the spring fish collections ranged from 14.3-16.6 °C depending on the sub-basin (Figure 16). Goshen Creek had the coldest median temperatures and State Farm had the warmest. The monthly high and low temperatures for the spring ranged from: Goshen (8.6-17.7°C), Flannery Fork (9.8-20.8°C), Winkler Creek (9.6-21.3°C), East Fork (9.0-21.8°C), Middle Fork (9.5-21.6°C), State Farm (9.5-22.5°C), Hodges Creek (9.4-20.6°C), and Boone Creek (10.3-21.5°C).

During the summer fish collections, the median monthly water temperature ranged from 16.7-19.1°C; where Goshen Creek remained the coolest and State Farm remained the warmest (Figure 15). The Dunn's Method comparison showed that these temperatures were statistically warmer compared to the spring based on P-values greater than 0.05. The monthly high and low temperatures for the summer ranged from: Goshen (13.5-18.5°C), Flannery Fork (15.3-21.6°C), Winkler Creek (15.1-22.0°C), East Fork (13.9-22.3°C), Middle Fork (14.2-22.2°C), State Farm (14.5-23.2°C), Hodges Creek (13.8-22.4°C), and Boone Creek (16.2-23.8°C).

For the summer collection the mean, greatest length of time, and percentage of the month that temperatures met or exceeded thermal stress for salmonids (20°C and above) for each sub-basin was determined. Goshen creek was the only sub-basin that did not exceed this temperature at any point in time during the summer collection month and Hodge's Creek did exceed the thermal stress temperature for a short duration (mean: 3.82 hours; longest duration: 6.75 hours) 35.48% of the days during the collection month (Table 6). The remaining sub-basins had temperature exceedances for more than 50% of the days in the collection month and the pulse temperatures stayed elevated for a longer duration with means ranging from 5.03-10.01 hours (Table 6).

The results of the relative abundances of cold-water fish species compared to median water temperature during the spring collection was not significant (R^2 = 0.164; P = 0.120), however, the summer collection was significant (P = 0.012) with a moderately strong R^2

value of 0.375 as shown by the linear regression analyses (Figure 17). These results reflect the relationships that were revealed by the PCA analysis which suggested that the percentage of cold-water fish abundances decreases as water temperature increases in the summer months. Alternately, the relative abundance of cool/warm water specialists did increase with temperature ($R^2 = 0.382$) which means that temperature may not be the only driver for cold-water species in the summer, increased competition between generalists and cold-water species may also influence the reduction of cold-water species in a site with warmer summer temperatures.

Individual species from the spring and summer were also compared to water temperature with statistically insignificant results. The coefficients of variation for the spring collection were: blacknose dace ($R^2 = 0.007$) and brown trout ($R^2 = 0.001$). The coefficients of variation for the summer collection were: blacknose dace ($R^2 = 0.051$) and brown trout ($R^2 = 0.059$). Water temperature was reducing overall cold-water fish numbers during the summer, but it was not affecting the distribution of any of the individual cold-water species. Regressions for brook trout, rainbow trout, and mottled sculpin were not used due to the lack of data for these species between sites.

The median monthly specific conductivity during spring fish collections ranged from 26.5-370.8 µS/cm depending on the sub-basin (Figure 18). The monthly high and low conductivity values for the spring ranged from: Goshen (13.5-40.2 µS/cm), Flannery Fork (19.0-38.2 µS/cm), Winkler Creek (16.4-34.2 µS/cm), East Fork (10.7-88.0 µS/cm), Middle Fork (33.2-96.7 µS/cm), State Farm (29.3-291.2 µS/cm), Hodges Creek (26.3-584.0 µS/cm), and Boone Creek (30.9-507.4 µS/cm). The median monthly specific conductivity during the summer fish collections had similar concentrations compared to the spring ranging from

34.6-319.1 µS/cm (Figure 18). Boone and Hodges Creeks held the highest concentrations during both the spring and summer collections. Winkler Creek had the lowest conductance in the spring and Flannery Fork had the lowest conductance in the summer. The monthly high and low conductivity values for the summer ranged from: Goshen (24.7-59.2 µS/cm), Flannery Fork (28.4-43.2 µS/cm), Winkler Creek (30.8-39.5 µS/cm), East Fork (28.9-119.5 µS/cm), Middle Fork (50.8-99.7 µS/cm), State Farm (50.5-185.4 µS/cm), Hodges Creek (42.0-219.6 µS/cm), and Boone Creek (18.6-501.2 µS/cm).

The spring cold-water fish species were compared to specific conductivity concentrations using a Spearman Correlation and linear regression with insignificant results ($P = 0.170$) (Figure 19). The relationship of individual species to conductivity was also statistically insignificant: blacknose dace ($P = 0.131$) and brown trout ($P = 0.115$). The summer results comparing relative abundances of cold-water fish to conductivity were also statistically insignificant ($P = 0.134$) (Figure 19). As was shown for the spring, the comparison of individual species to conductivity for the summer collections was also insignificant: blacknose dace ($P = 0.059$) and brown trout ($P = 0.052$). A positive correlation was expected between blacknose dace and conductivity for both sampling seasons per the PCA analysis results; this emphasizes the importance of additional statistical testing following a PCA analysis (Figure 12). Regressions for brook trout, rainbow trout, and mottled sculpin during both spring and summer were not used due to the lack of data for these species between sites. Conductivity, at least at the levels measured here, was not an influential variable on cold-water fish assemblages in the USFNR watershed.

Geographic Information System (GIS) Mapping

The calculated average riparian heights throughout the 150-m sampling transects ranged between 4.5-45.2 feet (Table 7). The reference sub-basins (Goshen, Flannery, Winkler) had noticeably taller vegetation (24.7-45.2 ft); the moderately (4.5-21.7ft) and highly impacted (5.8-12.8ft) sites were all under 22 ft. When the average riparian heights were compared to summer temperatures the relationship was poor (R^2 = 0.050) and the Pearson Correlation was not significant (P = 0.385) (Figure 19). However, when the heights were compared to conductivity the Pearson Correlation was significant (P = 0.013) and moderately strong with an R^2 of 0.369 (Figure 20).

The average riparian density percentages in the sampling transects ranged between 48.4-99.3% and there was not a comparable trend between sub-basin impact levels and the amount of vegetation within the 150-m sections (Table 6). The riparian categories ranged from poor to good, however, the reference sites did all score in the good category with percentages above 90% (Table 6). When the riparian density was compared to summer temperature using Pearson Correlation the regression was not significant (P = 0.321), however, when compared to conductivity the relationship was significant (P = 0.016) with an R^2 of 0.347 (Figure 21).

The percentage of impervious surfaces was calculated on a sub-basin level. The moderately impacted site State Farm (29.4%) had the highest percentage of impervious surfaces, followed by the highly impacted sub-basins Boone Creek (24.3%) and Hodge's Creek (14.4%); each reference site was under 5.0% (Figure 22). An accuracy assessment was performed in ArcMap by randomly generating 100 points and comparing the random points to their individual classification (0 = impervious; 1 = non-impervious) and ground truth within a 10-meter buffered layer of impervious surfaces. The resulting Confusion Matrix produced an overall accuracy percentage of 94.00% and a Kappa statistic of 85.98% which is very strong. The producer's accuracy for impervious classifications was 97.06% with 2.94% omission error and the user's accuracy was 94.29% with 5.71% commission error. The producer's accuracy for non-impervious classifications was 87.50% with 12.50% omission error and the user's accuracy was 93.33% with 6.67% commission error. This strengthens the results of the imperious surface layer because the impervious and non-impervious structures were overall correctly identified.

The percent of impervious surfaces was compared to the levels of conductivity within each sub-basin to determine the potential for run-off of contaminants that enter the waterway. The results of this regression were highly significant. The initial regression with all sub-basins included had a relatively strong R^2 value of 0.526, with an obvious outlier from the State Farm sub-basin (Figure 23). This outlier can be explained because State Farm is not only a larger order stream with a greater volume of water, it is also the site that represents the USFNR outlet, therefore all of the other sub-basins flow into it, which dilutes the conductivity (Table 1). The removal of the State Farm outlier produced a very strong regression ($R^2 = 0.972$) (Figure 23).

Lastly, the riparian density percentages, riparian heights, and percent of impervious surfaces were compared to relative cold-water fish abundances to determine any relationships using Spearman Rank Correlations and linear regressions. There was not a significant relationship between cold-water fish assemblages compared to riparian density in the spring ($P = 0.183$) or summer ($P = 0.135$). On the other hand, the relationship between cold-water fish and average riparian heights per sampling area was significant for the spring ($P = 0.006$)

and summer (P = 0.002) showing that the percent of cold-water fish does increase in sites with taller riparian vegetation (Figure 24).

The relationship between relative cold-water species abundance and sub-basin wide percentages of impervious surfaces was also significant in the spring (P = 0.004) and summer (P = 0.002) showing that cold-water fish negatively respond to increased amounts of impervious surface in the sub-basin (Figure 25).

Discussion

The purpose of this study was to identify the biogeophysical components that impact the cold-water fish assemblages within the USFNR watershed that uniquely contains headwater streams that are exposed to varying sub-basin habitats ranging from heavily urbanized areas with concentrated impervious surfaces to highly forested sub-basins that are protected by the Blue Ridge Parkway.

PCA Relationships

The PCA analysis looked for potential relationships between relative cold-water fish abundances and environmental variables with only relevant relationships being revealed. Riparian height, pebble count, conductivity, impervious surface percent, and BEHI were the variables that influence habitat quality and indicated potential relationships compared to cold-water fish species and each other in the USFNR, with addition of temperature in the summer results. In general, greater impervious surface percentage means that there is limited vegetation which directly contributes to an increased potential for contaminant runoff into the stream; the reduced vegetation also weakens the buffering capacity for conductivity, reduces shad and filtering of solar energy inputs, and fails to stabilize the banks. These inverse relationships suggested that the amount of cold-water fish increased in sites with colder water, less bank erosion, lower conductivity, and less impervious surface within the sub-basin. Additionally, greater riparian heights and better pebble count scores represent preferred habitat and the amount of cold-water fish did increase in response to these factors.

Riparian Corridors

The average riparian heights and densities did differ between sampling transects but did not have the thermal buffering impacts that were predicted on stream temperatures. This may be due to differences in land-use above the catchment points were the water quality sensors were collecting data. The Flannery Fork sub-basin was a reference site with tallest vegetation and riparian density above 95%, but it also had the second highest median water temperature during the summer compared to sites with poor vegetation. This warmer temperature may stem from the 16-acre Trout Lake, which is a shallow impoundment upstream from the sampling sites in Flannery Fork (Lord, 1981). Furthermore, a study by Poole & Berman (2001) listed phreatic ground water as high importance in the stabilization of stream temperatures in stream orders 1 & 2 and moderate importance in stream orders 3 & 4. Subsequent studies have also reported that riparian zones may have minimal effect on headwater streams due to groundwater inputs that stabilize the temperature (Cross *et al.*, 2013; Rutherford *et al.*, 2004).

Thermal Suitability

The monthly maximum temperature did surpass thermal limits (20.0-22.5 °C) for the salmonid species in 14 out 16 sites during the summer collections and cold-water abundances were lessened in response to temperature increases (Baldwin, 1957; Carlson *et al.*, 2017; Coutant, 1977; Elliot & Hurley, 2000; Wurtsbaugh & Davis, 1977). These temperature exceedances occurred over half of the days during the collection month in 6 out of 8 sites with the longest duration of temperature pulses ranging between 6.75-14.25 hours depending on the sub-basin. Thermal stress is known to affect the metabolic rates of cold-water fish resulting in reduced growth. The condition regressions that compared weight and length values for individual species revealed similar conditions in every species at every site despite the range of observed temperatures. Additionally, the plotted Fulton's condition factors for YOY and adult brown trout revealed similar fish conditions at every site despite variability in temperature, conductivity, instream habitat, and riparian vegetation. The YOY were expected to be particularly impacted due to their inability to seek thermal refuge like the adults with strong swimming ability. The similar fish conditions can be explained by the lab-to-field dilemma. There has been an abundance of studies that focus on pinpointing the thermal tolerances of individual salmonid species, but the majority of these studies are short-term lethal limit, laboratory conducted experiments that do not necessarily reflect real ecological scenarios of sub-lethal, long term effects on metabolism and growth (Selong *et al.*, 2001). Streams naturally have daily temperature fluctuations that most laboratory experiments fail to account for, instead most studies use constant temperatures or increase temperatures in a rapid manner to determine the critical thermal maximum limit (Wehrly *et al.*, 2007). It was also found that the trout were largely found in temperatures that exceeded 26.0°C and could tolerate temperatures that exceeded the recommended criteria for short periods of time (≤ 7 days) because the cold-water fish have adapted to cycling temperatures near the upper tolerances; this can cause stress to the fish but does not necessarily limit their persistence (Wehrly *et al.*, 2007). The maximum monthly temperatures did not reach 26°C at any time and the longest duration of elevated temperature was 14.25 hours in the USFNR watershed; water temperature was not high enough to cause significant stress or mortality. Although similar weight-length conditions were found between sites, the relative abundance of cold-water fish did decrease as temperatures increased. This could be due to the cold-water fish preferring lower temperatures or could be caused by the increased abundance of cool or warm water generalist species that compete for resources.

Conductivity

The median concentrations of specific conductivity were very different depending on the impact level of the sub-basin conditions. When these levels were compared to sampling transect riparian densities and heights the results were significant, but the R^2 values were not very strong indicating a weak correlation between riparian corridors of our sampling reaches and conductivity. It was also revealed that the height and density values are not dependent on each other because a transect can have high density vegetation that is not great in height (shrubs, tall grasses, etc.). The weak correlation is a result of the 150-m sampling transects failing to buffer out run-off contamination that occur upstream. Once conductivity was compared to the percentage of impervious surfaces on a sub-basin level, the relationship was very strong. The initially tested linear regression that compared conductivity and impervious surfaces for all sub-basins had a moderately strong relationship, with one obvious outlier.

This outlier was attributed to the State Farm sub-basin which had the highest percentage of impervious surfaces with only moderate concentrations of conductivity. This can be explained by the dilution paradigm "the solution to pollution is dilution" because State Farm is not only a larger order stream with a greater volume of water, it is also the outlet of the USFNR. When the linear regression was re-analyzed with the State Farm outlier removed the relationship was very strong (R^2 = 0.972) signifying that increases in impervious surfaces is a driver for higher concentrations of conductivity.

Conductivity was not an influential variable on cold-water assemblages or individual species during the spring or summer. Blacknose dace and brown trout are listed as intermediately tolerant to environmental pollution compared to intolerant rainbow trout or brook trout and were commonly found in higher levels of conductivity, but a significant relationship was not revealed in this study (NCDENR, 2013). The limited data for brook trout and rainbow trout that was collected in the USFNR headwaters makes it difficult to conclude whether environmental contaminants or physical barriers (weir dams, underground or clogged conduits) were limiting their prevalence in multiple sub-basins or if pressure from the more competitive brown trout were causing these limitations. Brown trout can outcompete other salmonids because they become piscivorous while brook trout and rainbow trout are mostly insectivorous. This can expand their range during periods of low productivity when terrestrial insects are less abundant. Observationally, brook trout and rainbow trout were also absent from Flannery Fork and Winkler sub-basins which maintain similar suitable conditions. Mottled sculpin are in the same tolerance class as blacknose dace and brown trout, but they were noticeably absent from accessible sub-basins with high concentrations of conductivity (Boone Creek and Hodge's Creek). The mottled sculpin were also absent from low conductivity sub-basin headwaters sites (Winkler Creek and Flannery Fork) but there is a Town of Boone water supply weir dam that physically blocks access to these sites and could explain their absence; unpublished data from the Ecotoxicology course taught by Dr. Shea Tuberty at Appalachian State University did find mottled sculpin at sites downstream of this barrier.

Overall, it was determined that water quality chemistry was not reducing the conditions of cold-water fish in the USFNR, but physical habitat conditions and summer temperatures were limiting distribution. Increased riparian height did not effectively reduce water temperature or runoff, but it did increase the percentage of cold-water fish found at those sites. Similarly, the amount of cold-water fish also increased in the presence of lower percentages of impervious surfaces within each sub-basin. The quality of bank and instream habitat also affected the abundances of cold-water species. The focal species negatively responded to increased stream bank erosion and benthic sedimentation.

Conclusions

The main focus of this study was to assess the cold-water fish species and determine which environmental and chemical variables were impacting the assemblages. The stream temperatures were not effectively buffered by riparian densities or heights within the transect zones, however, two of the sub-basins (Winkler Creek and Flannery Fork) were confounded by large upstream impoundments that may reduce the strength of this result. The amount of conductivity was effectively reduced compared to riparian corridors, but this correlation was much stronger using the comparison of impervious surfaces to conductivity on a sub-basin level because this accounts for the upstream runoff that is not included within the 150-m riparian corridor transects. The amount of impervious surface directly impacts the ability of contaminant run-off to enter a waterway. This highlights the importance of limiting any increasing impervious surfaces within the USFNR.

The ineffectiveness of the riparian corridors to reduce temperature may be attributed to differences in land-cover above the catchment points or ground-water inputs that create stability. Temperature over the range observed was not found to be an influential variable in the condition of cold-water fish species in the USFNR and the limited range of native brook trout and introduced rainbow trout is most likely attributed to competition with introduced brown trout. Conductivity may also reduce brook trout and rainbow trout prevalence, but the limited data that was collected for these species during this study is not enough to draw any conclusions. Rainbow trout and brook trout are insectivores and conductivity may be indirectly driving assemblages; previous research by Fleetwood, 2017 found that aquatic macroinvertebrates react negatively to increased conductivity. A field study by the Environmental Protection Agency (EPA) reinforces this by listing the freshwater aquatic life benchmark for invertebrate genera in Central Appalachian streams at 300µS/cm (EPA, 2011). The collection of rainbow trout and brook trout from additional sites is needed to determine if conductivity is a driving factor. Nevertheless, this study does produce valuable insight for future work. It is rare for sustainable assemblages of naturalized trout in multiple year classes to be found in headwater streams that are nestled within a unique watershed with sub-basins that are exposed to vastly different habitats that range from heavily urbanized areas as well as the highly forested and federally protected Blue Ridge Parkway.

Although water temperature was revealed by the PCA as an important factor in only the summer collection for distribution, it was not found to influence cold-water fish conditions over the observed range of temperatures, however; physical habitat factors (stream bank erosion, benthic substrate, riparian forest cover height, and impervious surfaces) did influence cold-water fish distributions throughout the USFNR. The assemblages of cold-water fish increased with quality bank and instream habitat which emphasizes the importance for state and local agencies to continue to preserve or improve habitat conditions to maintain or restore cold-water species persistence. Subsequently, taking steps to protect these streams from the factors that degrade physical habitat will also aid in the conservation of cold-water assemblages in the future when climate change is predicted to increase thermal stress further.

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Tables and Figures

Table 1 Table listing the sub-basin, impact rating, and geographical coordinates of each sampling site.

SITE	Sub-Basin	Stream Order	Impact Level	Latitude	Longitude
GC-DC	Goshen	3	Reference	36.181325	-81.609645
GC-SD	Goshen	3	Reference	36.183335	-81.610669
WC-PP	Winkler	2	Reference	36.184894	-81.678041
WC-SD	Winkler	2	Reference	36.195532	-81.678162
FF-SD	Flannery	2	Reference	36.182521	-81.686798
FF-HS	Flannery	2	Reference	36.182746	-81.686762
MF-TW	Middle Fork	3	Moderate	36.167766	-81.647322
MF-KT	Middle Fork	3	Moderate	36.193784	-81.651123
EF-BR	East Fork	3	Moderate	36.192753	-81.635206
EF-SD	East Fork	3	Moderate	36.202627	-81.648967
SF-TW	State Farm	5	Moderate	36.201961	-81.652219
SF-SD	State Farm	5	Moderate	36.208869	-81.654515
HC-SB	Hodges	2	Highly	36.202721	-81.672287
HC-SD	Hodges	2	Highly	36.202347	-81.66999
BC-SD	Boone	3	Highly	36.211889	-81.678211
BC-AMB	Boone	3	Highly	36.204572	-81.669213

*Reference = low conductivity; moderate = intermediate conductivity; highly = high conductivity

Table 2 Table listing Bank Erosion Hazard Index (BEHI) categories and scores used to rank the erodibility of a stream bank.

BEHI Category	Root Depth	RDH Score	Root Density	RD Score	Surface Protection	SP Score	Bank Angle	BA Score
Very Low	90 - 100	1	80 - 100	1	80 - 100	1	0 - 20	1
Low	50 - 89	3	55 - 79	3	55 - 79	3	21 - 60	3
Moderate	30 - 49	5	30 - 54	5	30 - 54	5	61 - 80	5
High	15 - 29	7	15 - 29	7	15 - 29	7	81 - 90	7
Very High	5 - 14	8.5	5 - 14	8.5	10 - 14	8.5	91 - 119	8.5
Extreme	< 5	10	< 5	10	< 14	10	> 119	10

*Combined BEHI Scores: ≤ 6 = Very Low; 6-12 = Low; 13-20 = Moderate; 21-28 = High; 29-34 = Very High; > 34 = Extreme

Table 3 Location of Eureka Manta 20+ and Trimeter water quality sondes in the USFNR headwater streams.

Stream Name	Sensor Type	Latitude (N)	Longitude (W)
Boone Creek	Eureka Manta 20+	36° 12’42.7	81° 40’34.0
East Fork	Eureka Manta 20+	36°12’08.5	81°38’54.6
Flannery Fork	Eureka Manta 20+	36°11’05.7	81°40’41.6
Goshen Creek	Eureka Manta 20+	36°11’00.5	81°36’38.5
Hodges Creek	Eureka Trimeter	36°12’08.5	81°40’12.1
Middle Fork	Eureka Manta 20+	36°11’29.2	81°39’18.2
State Farm	Eureka Manta 20+	36°12’30.7	81°39’12.5
Winkler Creek	Eureka Trimeter	36°11’43.2	81°40’51.8

Table 4 Breakdown of relative percent focal species abundance and relative percentages of functional feeding groups by sampling site for spring and summer collections (GC/FF/WC = reference; EF/MF/SF = moderately impacted; HC/BC = highly impacted).

Spring Sites	Relative % Focal Species Abundance	% Insectivore	% Herbivore	% Piscivore	% Omnivore
GC-DC	100.00	52.94	0.00	47.06	0.00
GC-SD	99.07	67.59	0.00	32.41	0.00
FF-SD	68.75	46.88	0.00	53.13	0.00
FF-HS	100.00	15.15	0.00	84.85	0.00
WC-PP	45.45	90.91	0.00	9.09	0.00
WC-SD	87.36	60.92	0.00	39.08	0.00
EF-SD	32.28	89.76	8.66	1.57	0.00
EF-BR	37.16	77.03	15.54	4.73	2.70
MF-TW	54.49	65.27	25.15	9.58	0.00
MF-KT	26.50	69.23	22.22	4.27	4.27
SF-TW	2.38	60.00	21.25	17.50	1.25
SF-SD	22.53	78.57	17.03	2.75	1.65
HC-SB	60.87	60.87	1.45	33.33	4.35
HC-SD	38.71	54.84	30.65	11.29	3.23
BC-SD	48.45	51.55	45.36	0.00	3.09
BC-AMB	31.07	65.05	29.61	4.85	0.49

Summer Sites	Relative % Focal Species Abundance	% Insectivore	% Herbivore	% Piscivore	% Omnivore
GC-DC	100.00	28.57	0.00	71.43	0.00
GC-SD	83.61	81.15	0.00	18.85	0.00
FF-SD	76.27	45.76	0.00	54.24	0.00
FF-HS	83.67	26.53	0.00	73.47	0.00
WC-PP	63.89	91.67	0.00	8.33	0.00
WC-SD	82.19	60.27	0.00	39.73	0.00
EF-SD	59.57	83.51	4.79	11.70	0.00
EF-BR	26.79	63.64	21.53	9.57	5.26
MF-TW	49.18	59.84	27.87	12.30	0.00
MF-KT	39.66	74.30	18.44	7.26	0.00
SF-TW	15.01	73.37	20.40	4.53	1.70
SF-SD	21.39	80.92	17.34	1.73	0.00
HC-SB	65.66	66.67	1.01	30.30	2.02
HC-SD	65.08	88.89	1.59	7.94	1.59
BC-SD	63.25	75.21	24.79	0.00	0.00
BC-AMB	19.35	77.42	12.90	0.00	9.68

Table 5 Table listing the Wolman Pebble Count scores (PC Score), category (PC Category), and D_{50} values by site and sub-basin

SITE NAME	Sub-Basin	PC Score	PC Category	D_{50}	D_{50} Category
MF-KT	Middle Fork	3.73	Good	3-64 mm	2
MF-TW	Middle Fork	3.39	Good	3-64 mm	2
HC-SD	Hodge's Creek	2.81	Marginal	0.62-2 mm	1
HC-SB	Hodge's Creek	2.98	Marginal	0.62-2 mm	1
BC-SD	Boone Creek	3.42	Good	3-64 mm	2
BC-AMB	Boone Creek	2.84	Marginal	0.62-2 mm	1
WC-PP	Winkler Creek	3.69	Good	3-64 mm	2
WC-SD	Winkler Creek	3.69	Good	3-64 mm	2
FF-SD	Flannery Fork	3.99	Good	3-64 mm	2
FF-HS	Flannery Fork	3.77	Good	3-64 mm	2
EF-BR	East Fork	2.67	Marginal	0.62-2 mm	1
EF-SD	East Fork	3.22	Good	3-64 mm	2
GC-SD	Goshen Creek	3.93	Good	3-64 mm	2
GC-DC	Goshen Creek	3.83	Good	3-64 mm	2
SF-SD	State Farm	3.01	Good	3-64 mm	2
SF-TW	State Farm	2.68	Marginal	0.62-2 mm	1

* D_{50} scores categorized as: 0.62-2mm = 1; 3-64mm = 2; 65-255mm = 3; 256-1096mm = 4

Table 6 List of individual sub-basins and the mean time (hrs), greatest time (hrs), and percentage of the month that water temperatures exceeded 20°C.

Sub-Basin	Mean Time (hrs) above 20°C	Greatest Time (hrs) above 20°C	% of Month
Goshen Creek	0.00	0.00	0.00
Flannery Fork	9.54	13.50	58.06
Winkler Creek	5.03	7.50	51.61
East Fork	8.56	11.25	58.06
Middle Fork	7.65	12.75	67.74
State Farm	10.01	14.25	77.42
Hodge's Creek	3.82	6.75	35.48
Boone Creek	5.83	9.25	67.74

Table 7 Comparison of average riparian height (ft), average density percentages, and categories for each 150-m sampling transect within USFNR watershed. GC/FF/WC = reference; EF/MF/SF = moderately impacted sub-basin; HC/BC = highly impacted sub-basin.

Site	Riparian Height	Riparian %	Riparian Category
GC-DC	31.4	99.3	Good
GC-SD	31.8	90.8	Good
FF-SD	45.2	96.8	Good
FF-HS	37.7	95.2	Good
WC-PP	34.6	98.5	Good
WC-SD	24.7	71.5	Fair
EF-SD	16.7	63.7	Fair
EF-BR	4.5	75.2	Fair
MF-TW	5.7	84.1	Good
MF-KT	21.7	98.2	Good
SF-TW	7.9	77.2	Fair
SF-SD	9.6	80.1	Good
HC-SB	12.8	89.5	Good
HC-SD	15.8	94.0	Good
BC-SD	5.8	48.4	Poor
BC-AMB	10.4	59.7	Fair

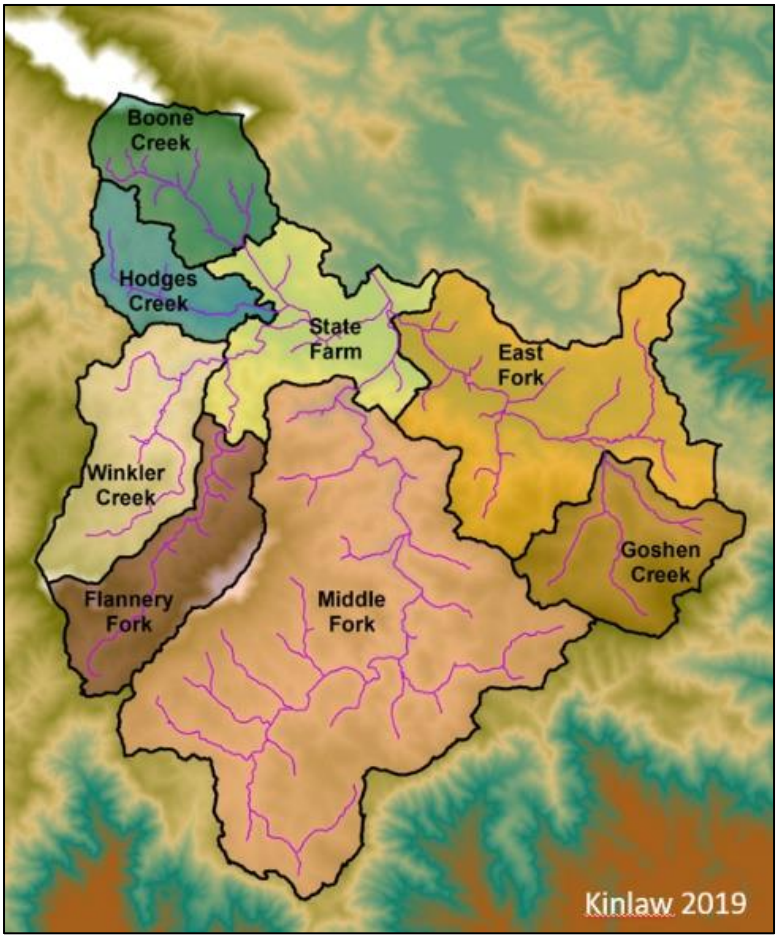


Fig. 1 Map showing the Upper South Fork New River watershed and associated sub-basins in Watauga County, NC.

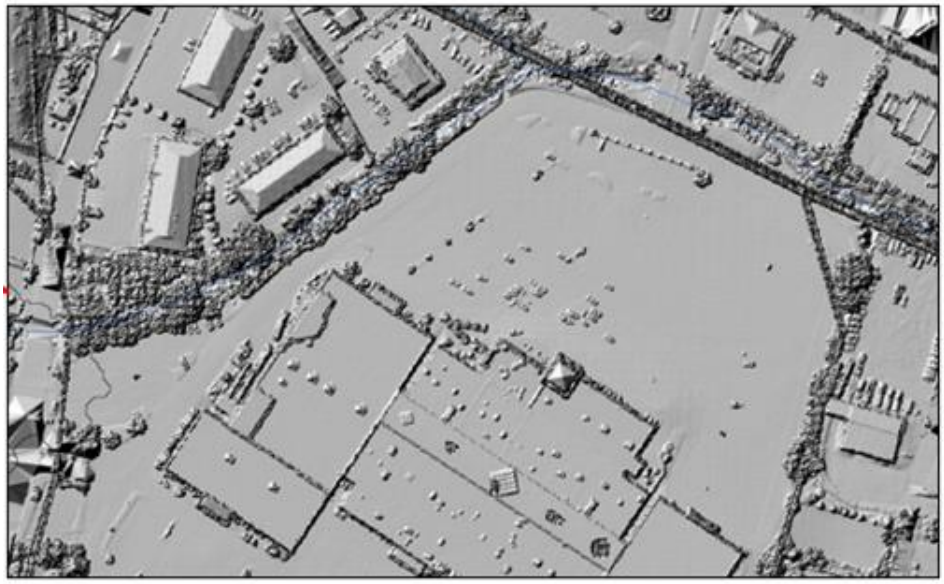


Fig. 2 Hillshade representation of the vegetation layer of the 2018 LiDAR-Geiger data at HC-SD sampling site, adjacent to the Boone Mall parking lot in Watauga County, NC.

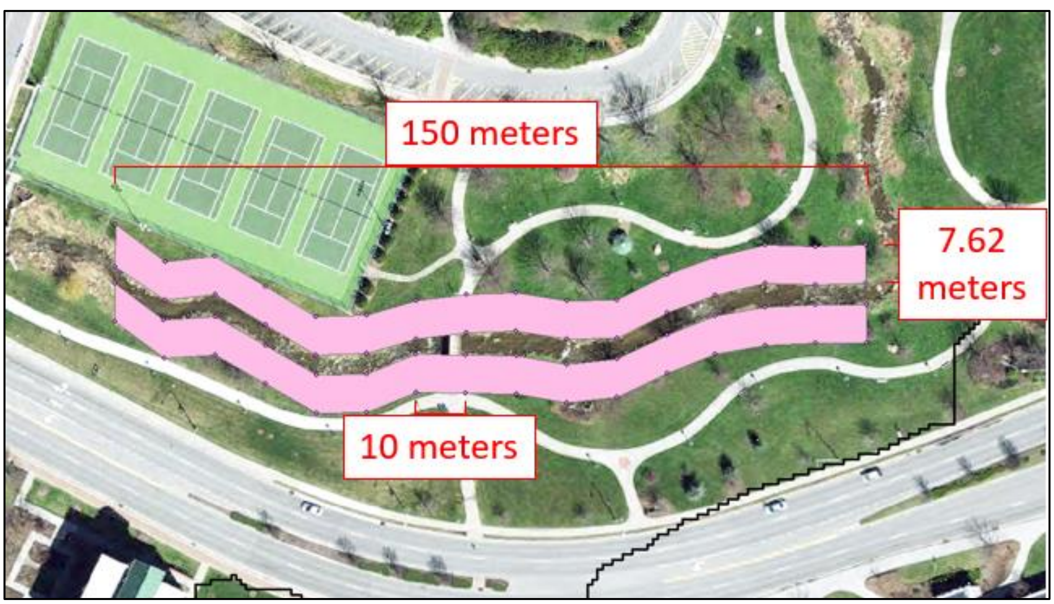


Fig. 3 ArcMap figure of the connected multipoints drawn every 10-meters that form the polygon features that represent riparian corridor buffers within the sampling transect.

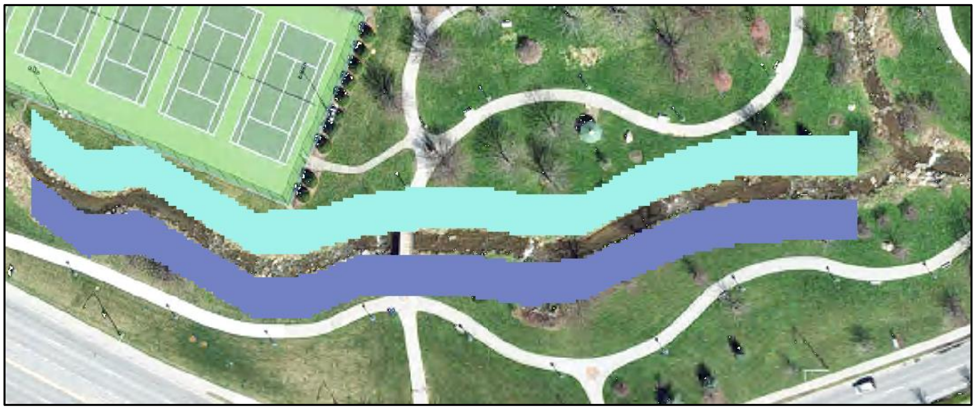


Fig. 4 Result of the Feature to Raster tool which converted each of the created polygon features into rasters that can be used to calculate riparian height and density statistics within the sampling areas. Here is one of the transects on Boone Cr on the ASU campus.

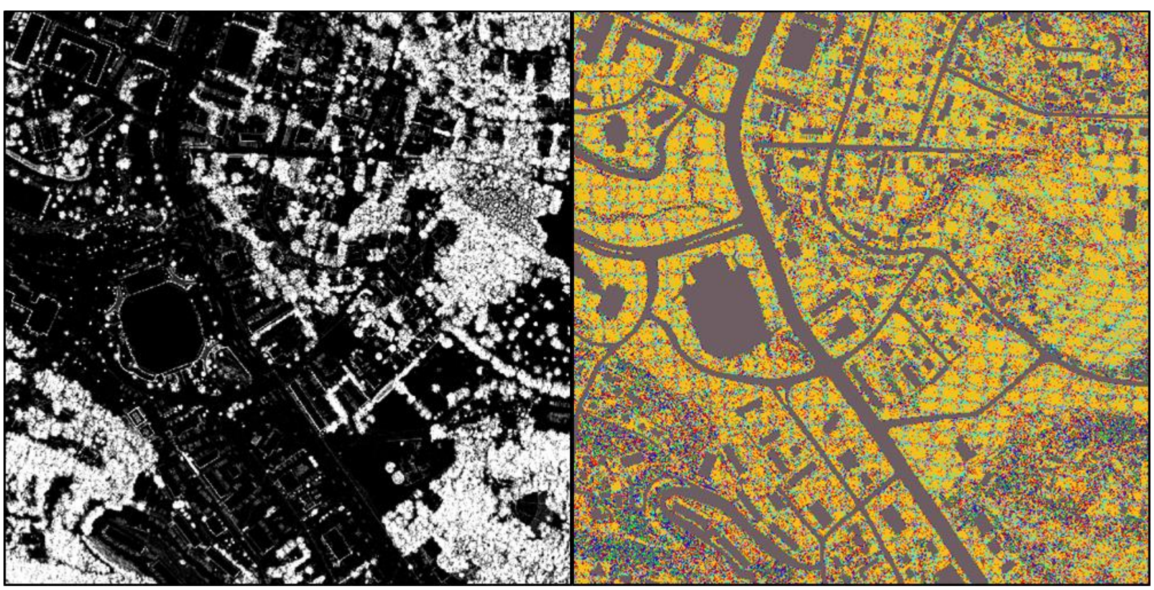


Fig. 5 Left-.LAS Point Statistics as Raster for vegetation layer; right-.LAS Point Statistics as Raster for bare-earth layer.

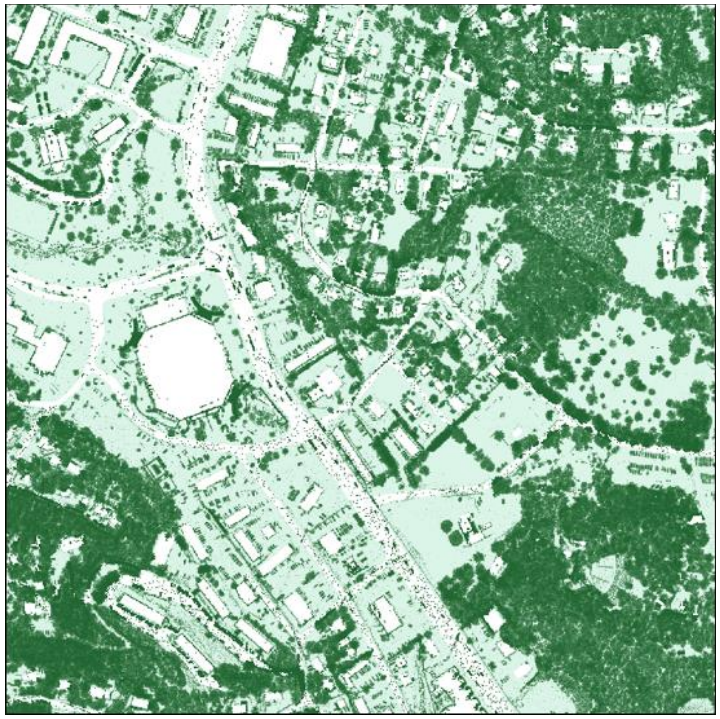


Fig. 6 Model of canopy density; darker areas indicate higher vegetative density values.

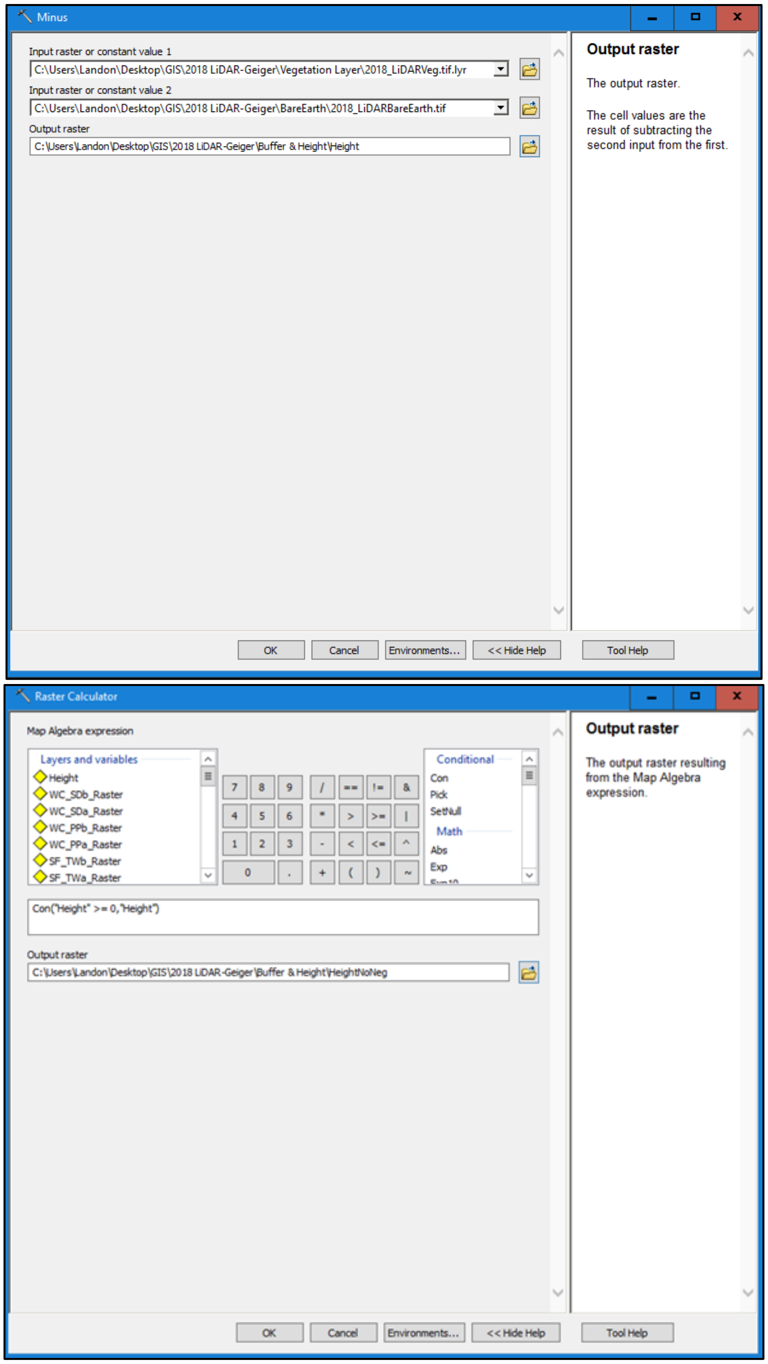


Fig. 7 Minus tool (top) and Raster Calculator (bottom) in ArcMap 10.5; calculates the differences in height between LiDar-Geiger vegetation layers and LiDar-Geiger bare-earth layers.

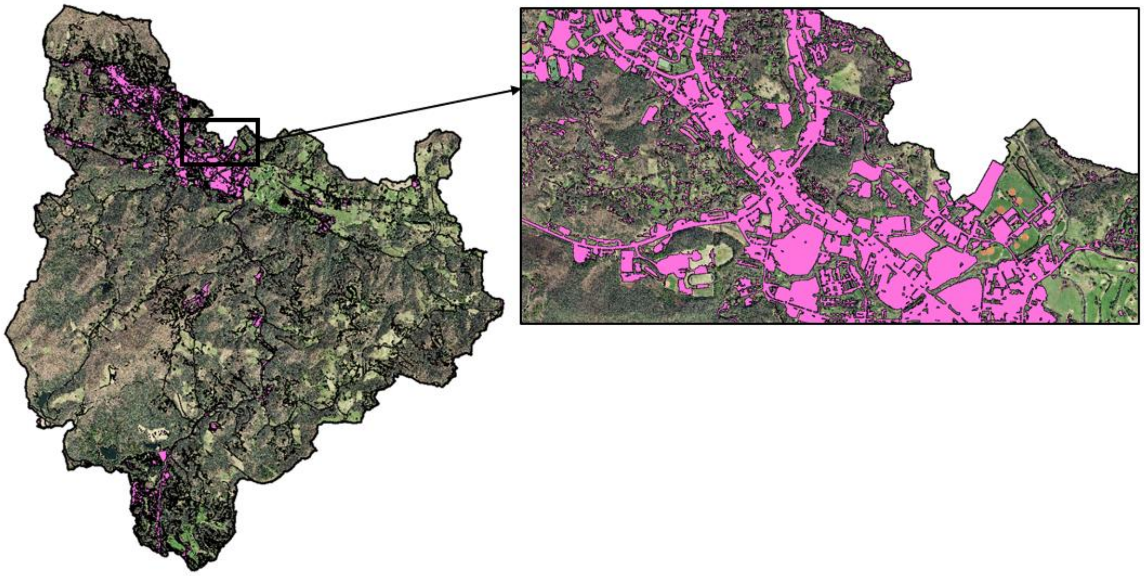
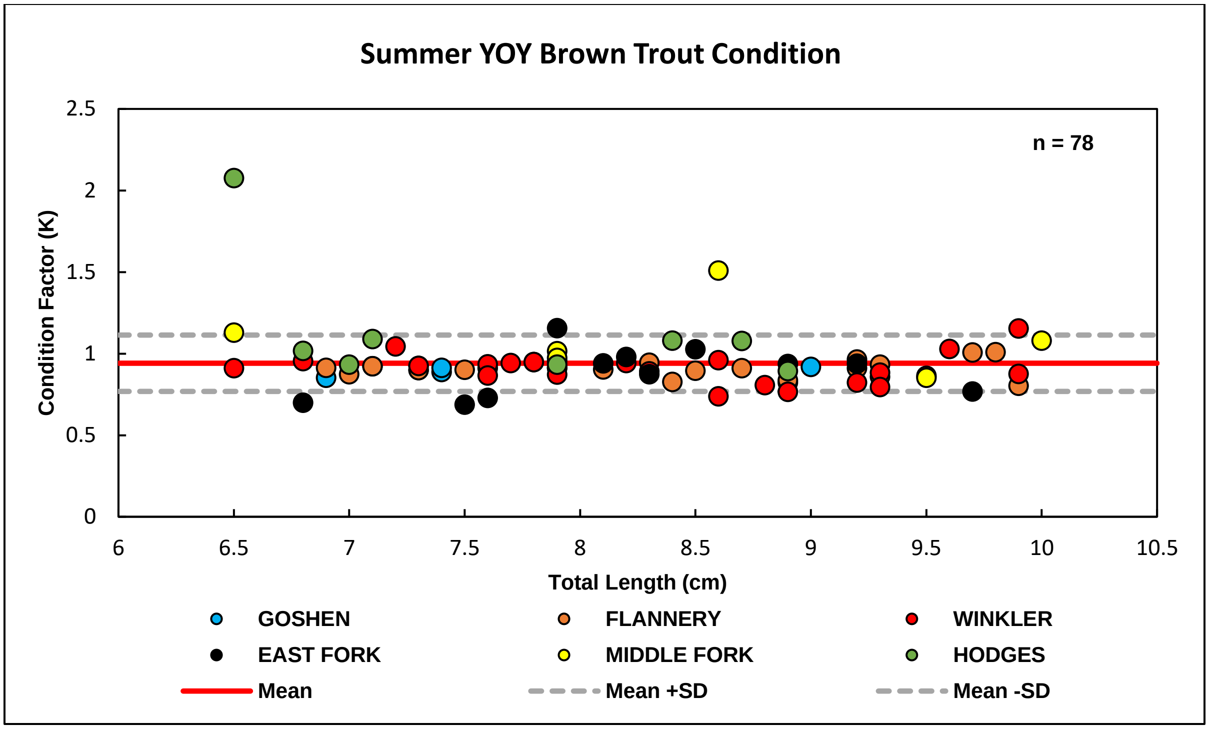


Fig. 8 Resulting impervious surface layer created using Supervised Learning tool in the Feature Analyst extension; pink polygons indicate impervious surfaces that were identified using the initial learning algorithm.

Fig. 9 Examples of missed or misidentified features that were corrected from the Feature Analyst output shapefile during the manual editing process. Brown and red roofs were often missed (yellow boxes show missed features) and portions of field were misidentified as impervious surface.



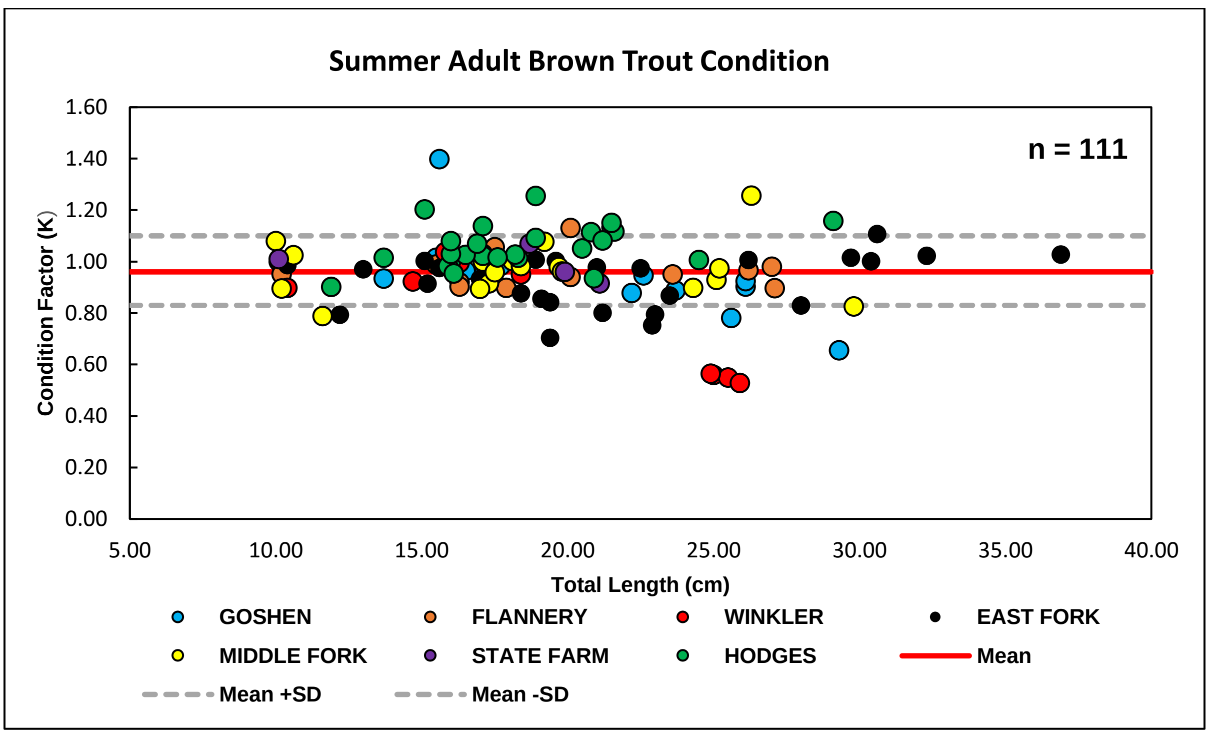


Fig. 10 Plotted Fulton Condition Factor (K) and total length (cm) for YOY and adult brown trout during summer collections; points are color-coded by sub-basin to determine condition patterns.

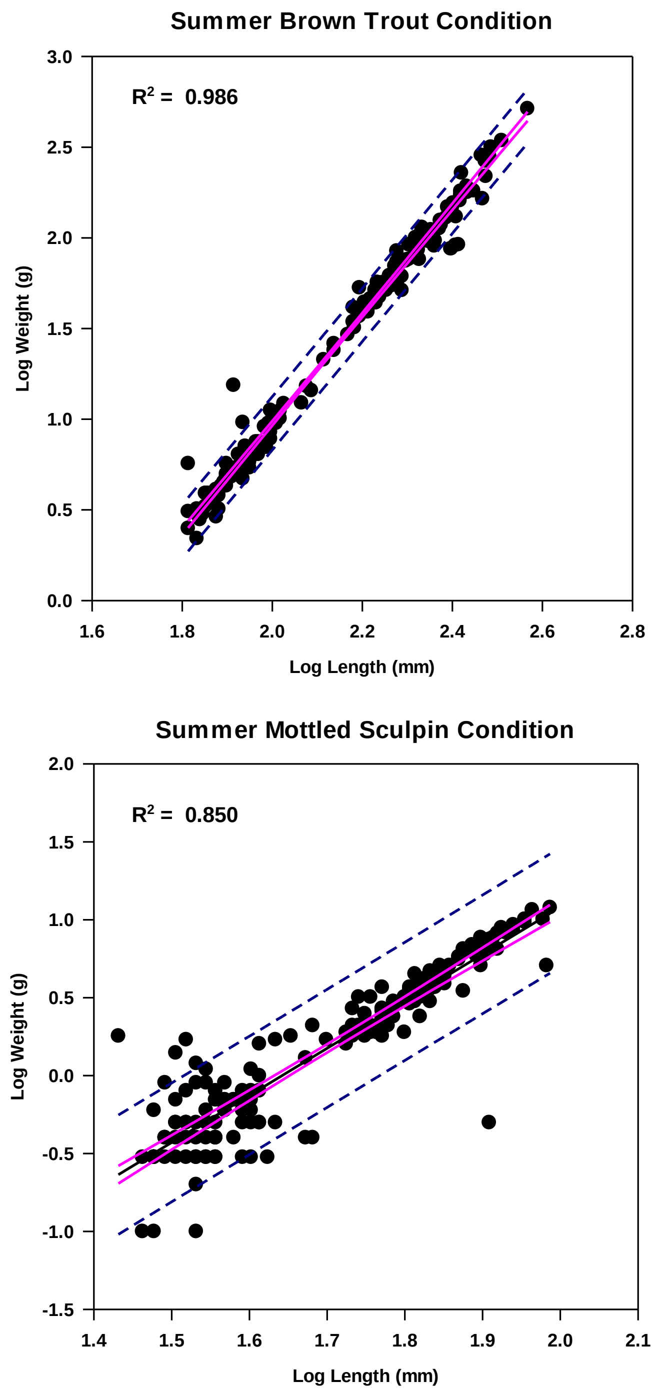


Fig. 11 Regression comparison of log weight (g) and length (mm) measurements for the summer fish collection to determine fish conditions between cold-water species; blue line indicates 95% predicted values and pink line represents the 95% confidence interval (P= < 0.001 all regressions).

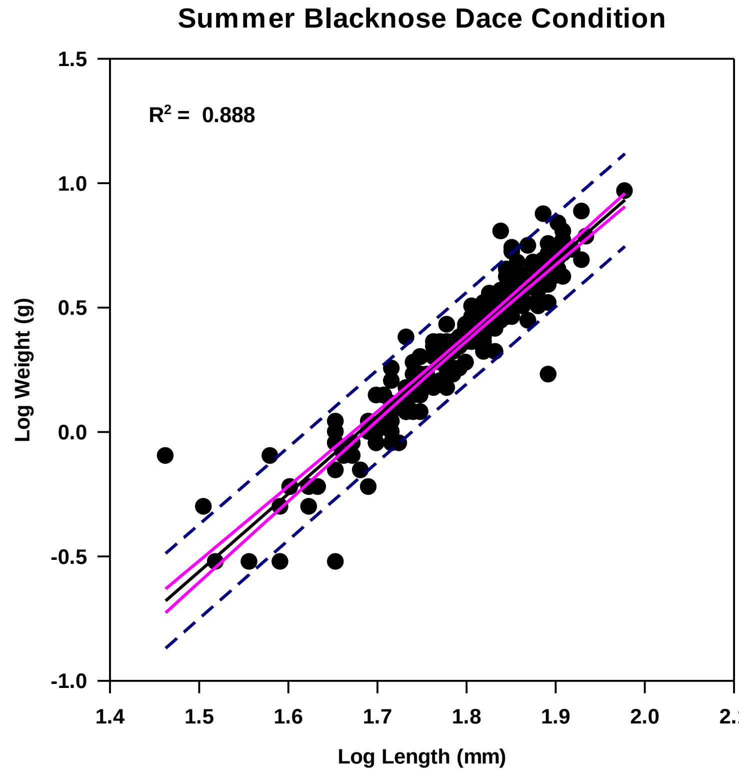


Fig. 11 Continued Regression comparison of log weight (g) and length (mm) measurements for the summer fish collection to determine fish conditions between cold-water species; blue line indicates 95% predicted values and pink line represents the 95% confidence interval (P= < 0.001 all regressions).

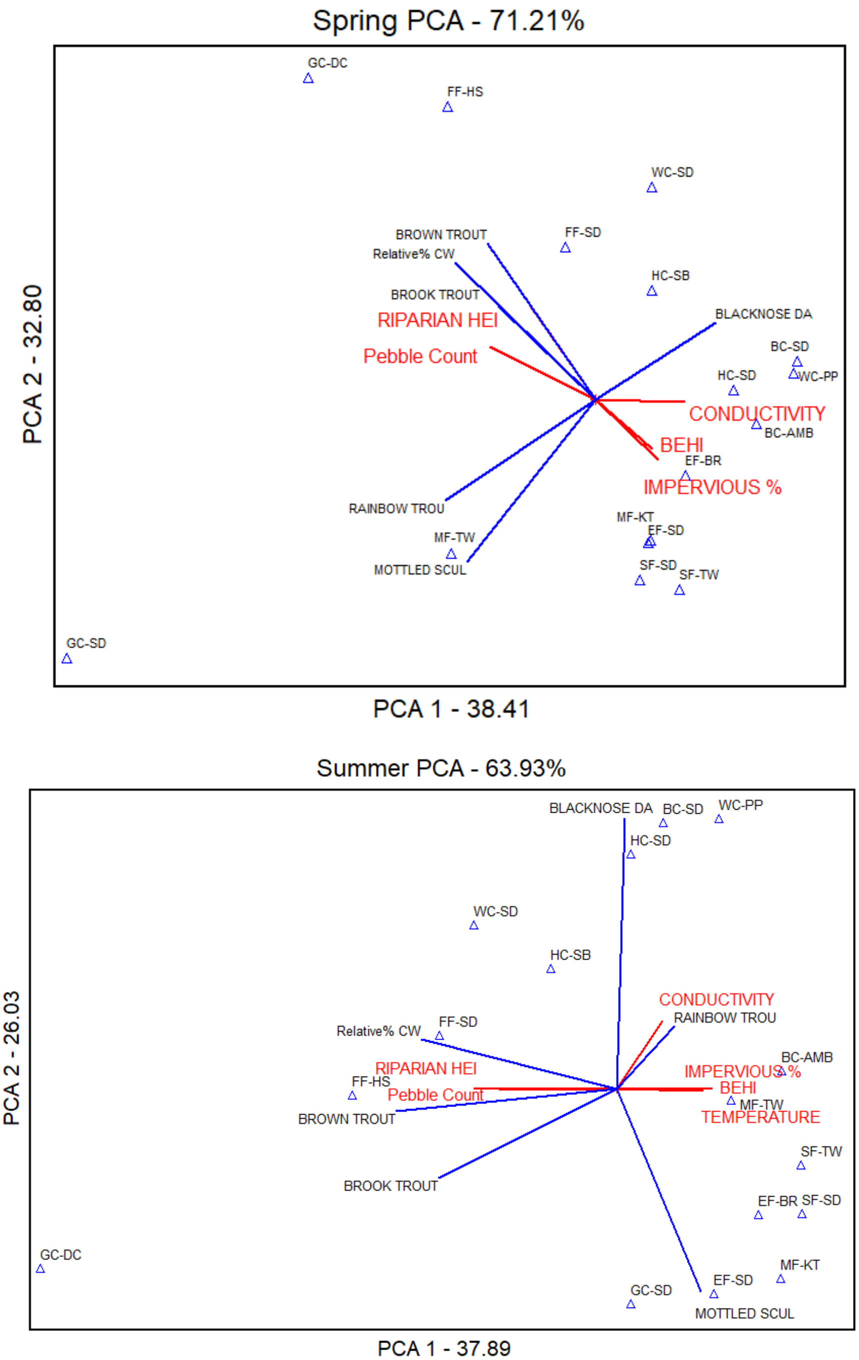
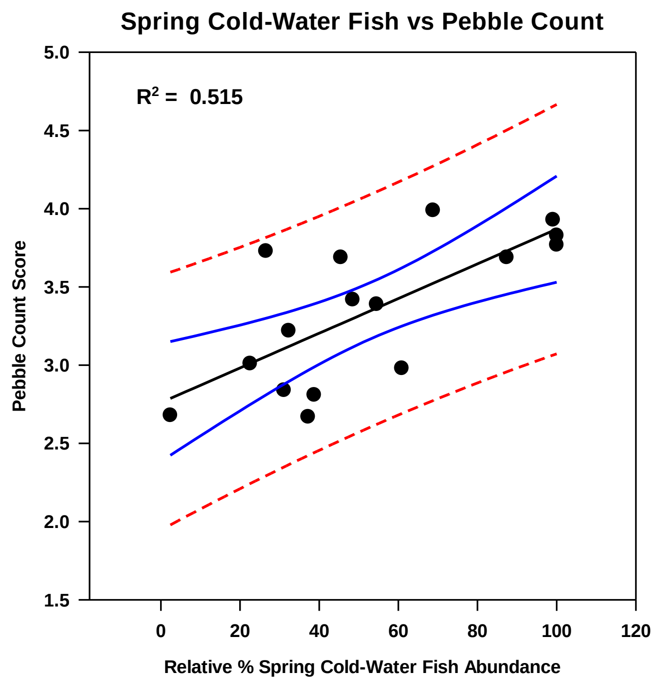


Fig. 12 Joint-Plot Principle Components Analysis (PCA); of Spring and Summer collections; Spring PCA accounts for 71.21% of variation within the dataset and the Summer accounts for 63.93%; triangles represent study sites, blue vector lines represent fish species, and red vector lines represent relevant environmental variables.



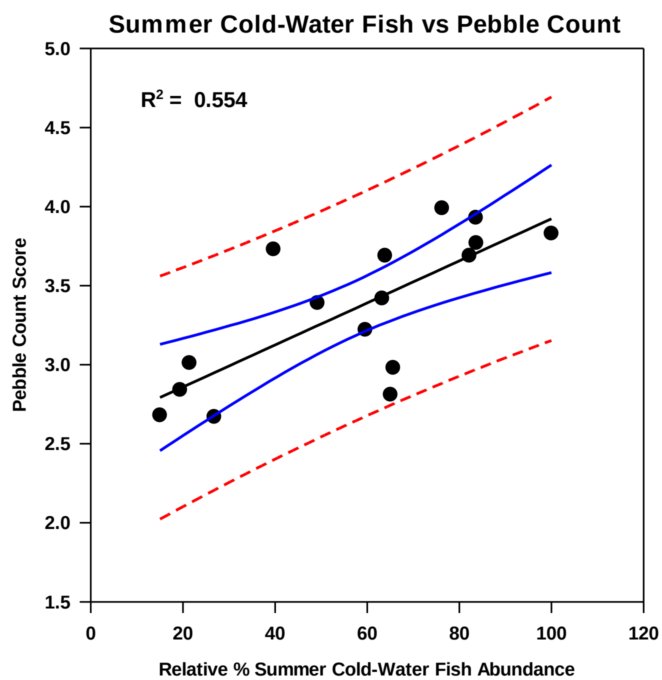


Fig. 13 Regression analysis of the Wolman Pebble Count Scores compared to the relative percentage of cold-water fish abundances for the spring (top; $P = 0.002$) and summer (bottom; $P \leq 0.001$) collection; dashed blue line represents 95% confidence interval and pink line represents 95% predicted values.

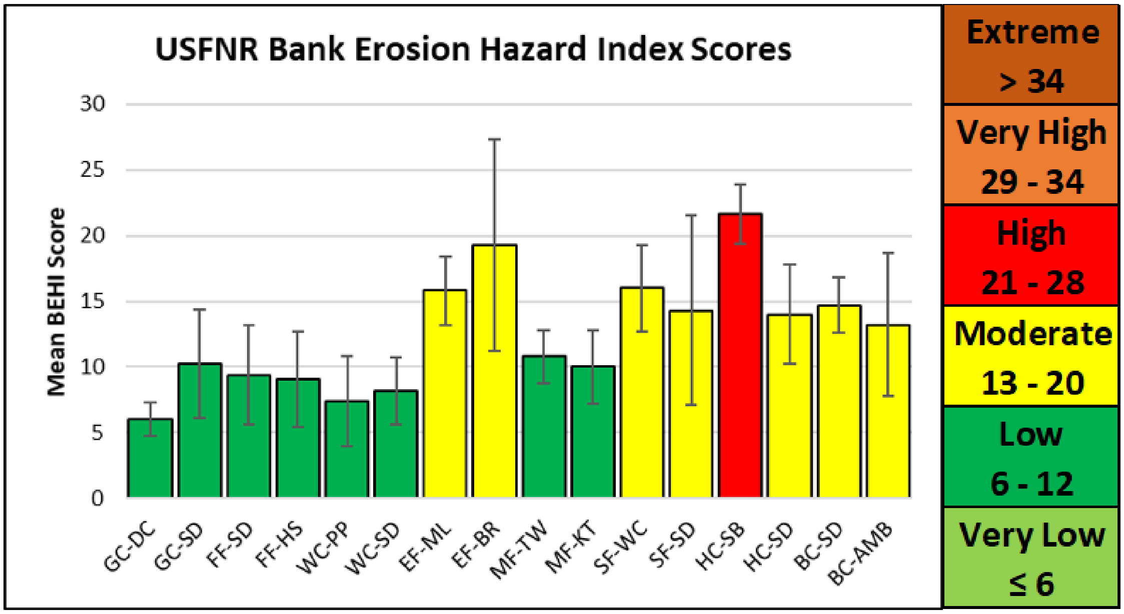


Fig. 14 Graphical representation of the mean Bank Erosion Hazard Index (BEHI) scores ± 1SD at each site within the Upper South Fork New River (USFNR) watershed; green bars represent low, yellow bars represent moderate, and red bar represents high potential for stream bank erosion.

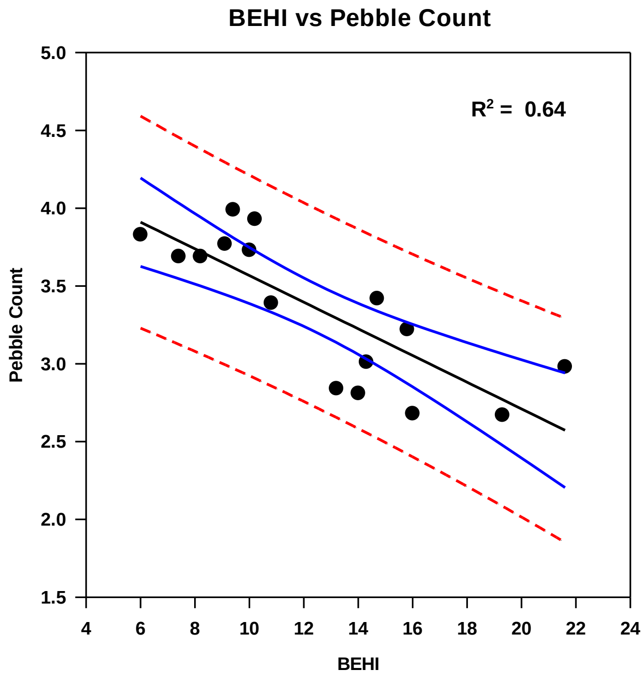


Fig. 15 Regression comparison of Wolman Pebble Count scores and Bank Erosion Hazard Index (BEHI) scores; blue line represents 95% confidence interval and red line represents 95% predicted values ($P \leq 0.001$).

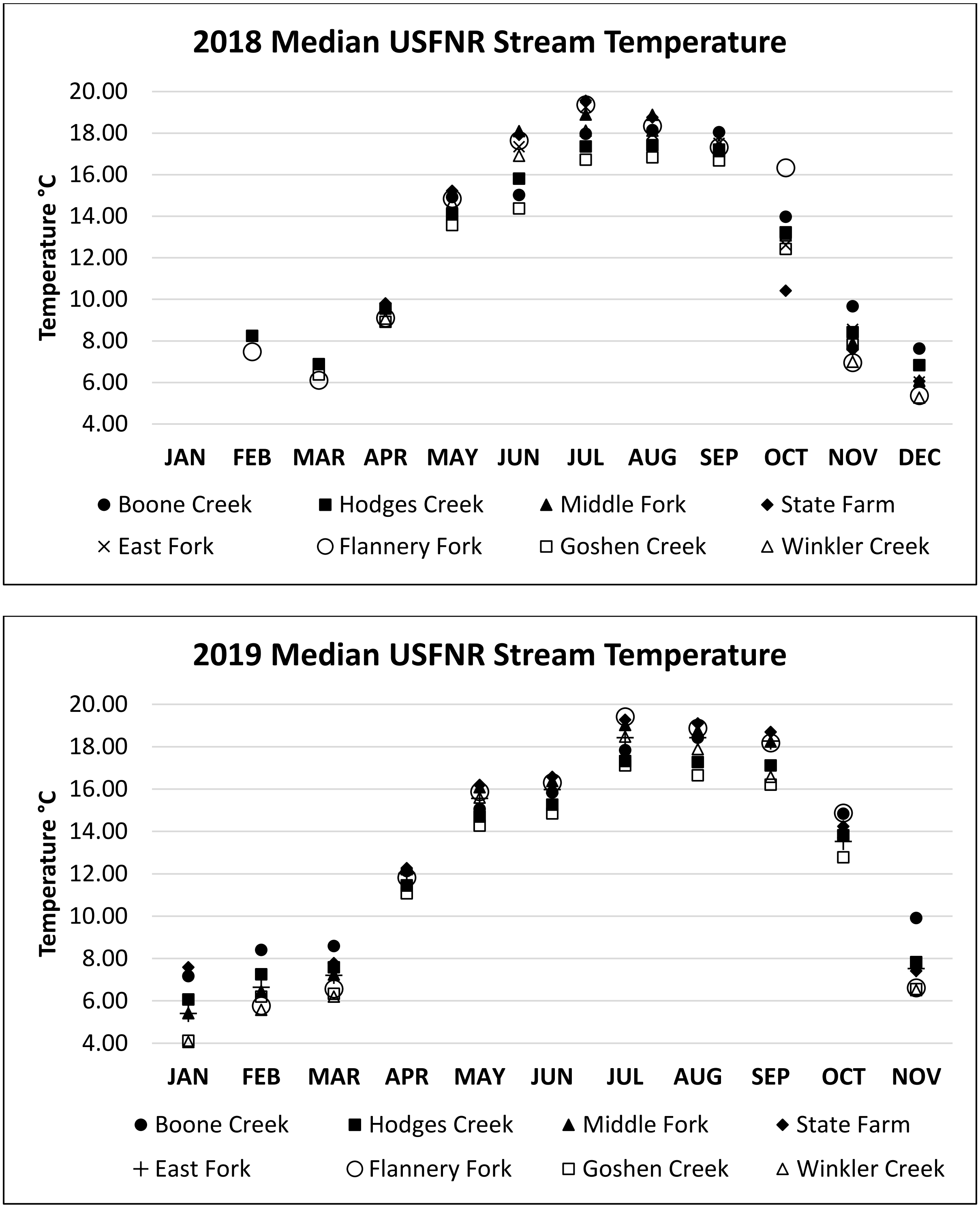
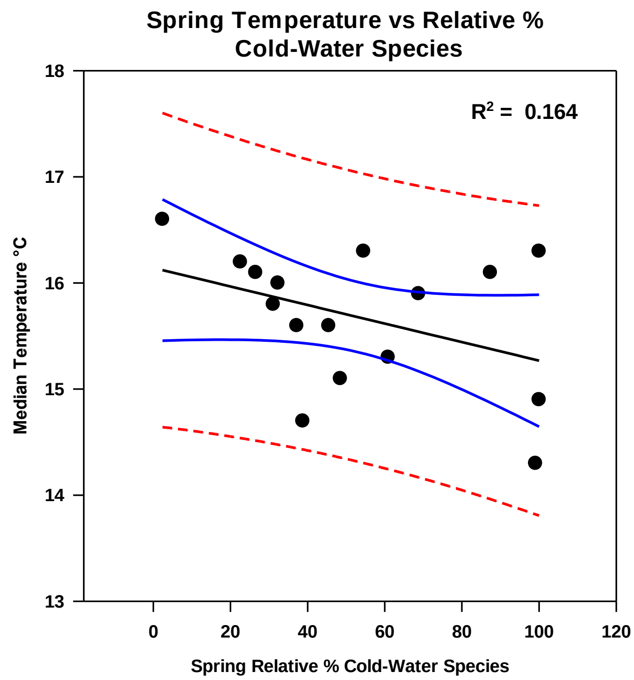


Fig. 16 Median monthly water temperatures for each sub-basin within the Upper South Fork New River (USFNR) watershed for 2018 and 2019.



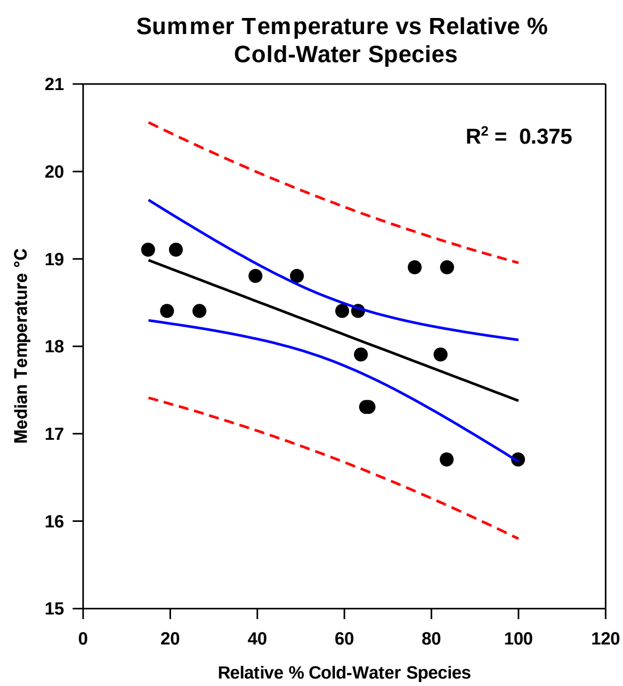
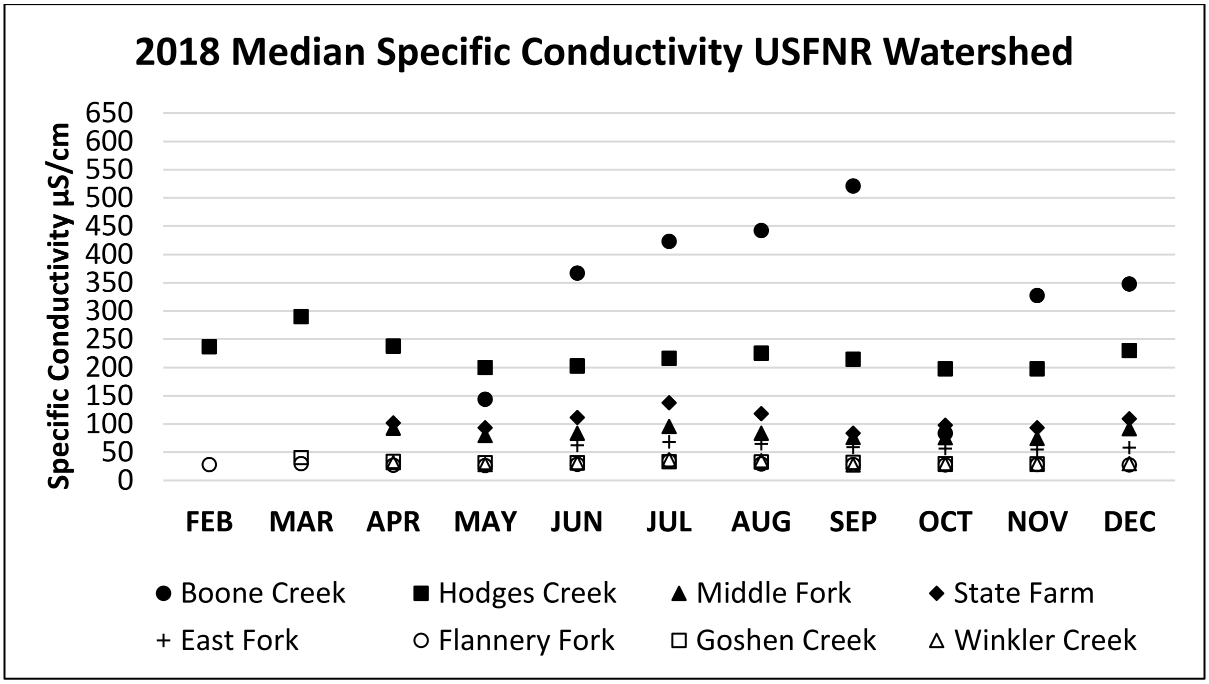


Fig. 17 Linear regression of relative percentage of cold-water species abundance compared to median water temperatures for both spring (top; P = 0.120) and summer (bottom; P = 0.012) collections; red line indicates 95% predicted values; blue line represents 95% confidence interval; black line indicates regression.



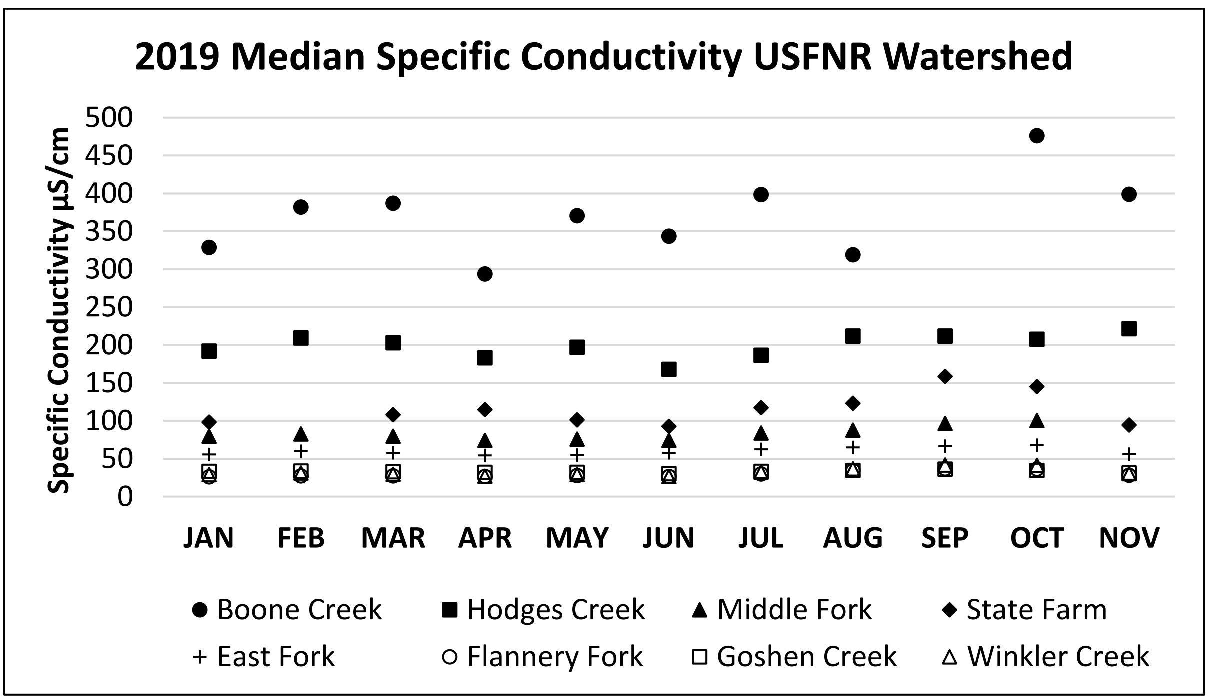
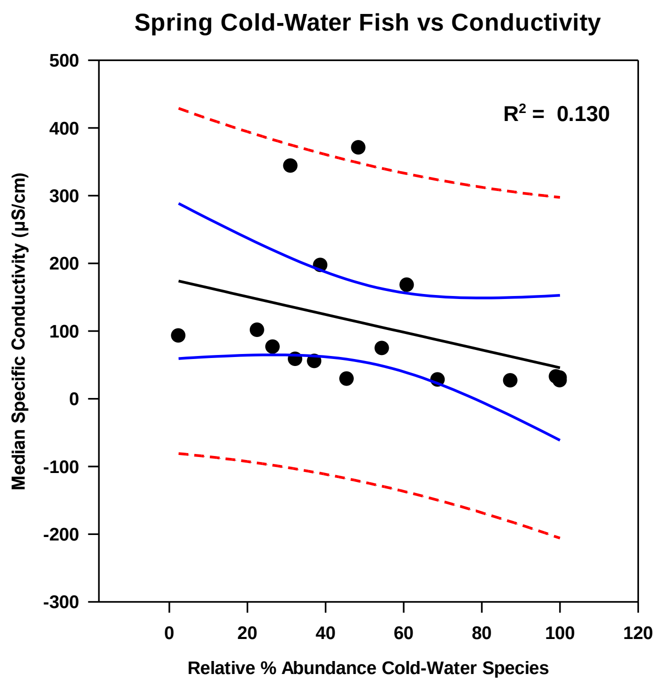


Fig. 18 Median monthly specific conductivity values for each sub-basin within the Upper South Fork New River (USFNR) watershed for 2018 and 2019.



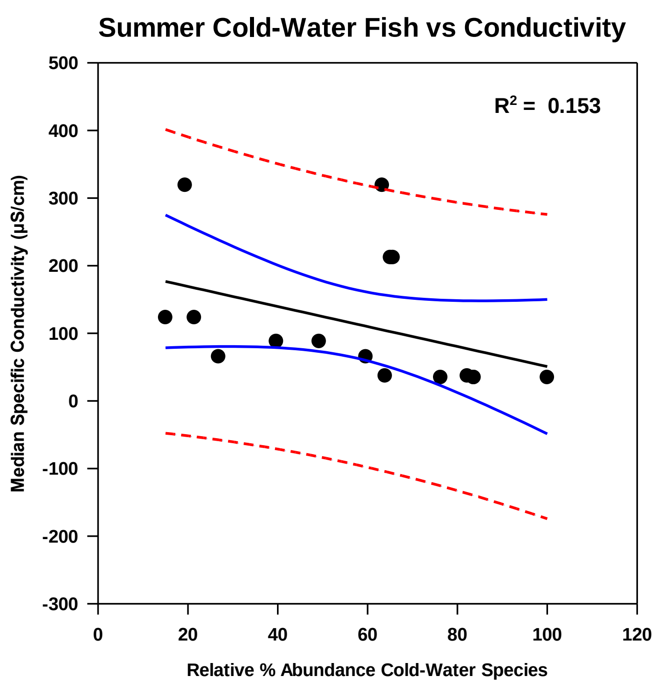
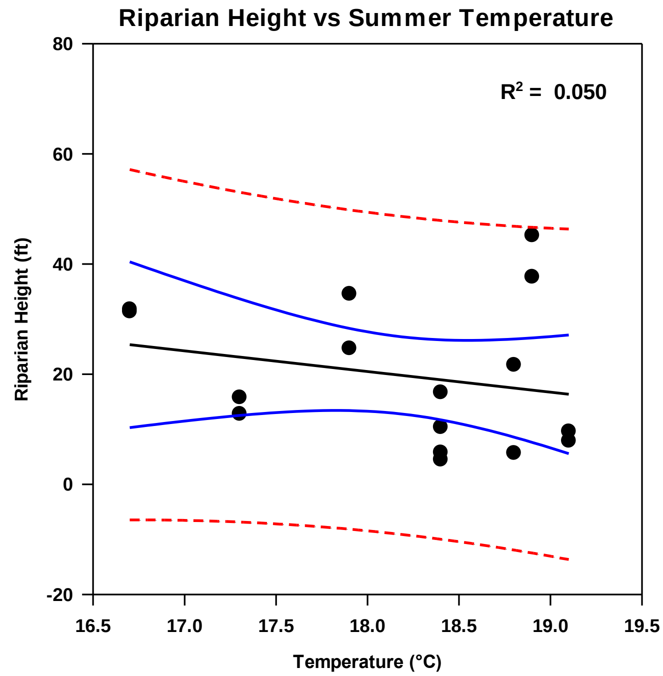


Fig. 19 Regression analysis of relevant abundances of cold-water fish species compared to concentrations of specific conductivity (µS/cm) during spring (top; P = 0.170) and summer (bottom; P = 0.134) collections. Red line indicates 95% predicted values; blue line indicates 95% confidence interval; black line represents regression.



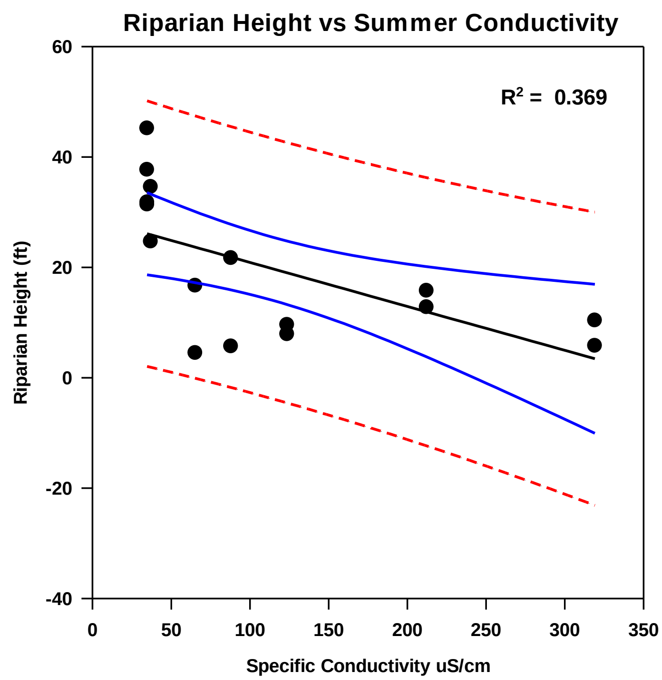


Fig. 20 Top: regression of riparian height (ft) compared to median summer temperatures (°C) ($P = 0.385$); bottom: regression of riparian height (ft) compared to median specific conductivity (μS/cm) ($P = 0.013$).

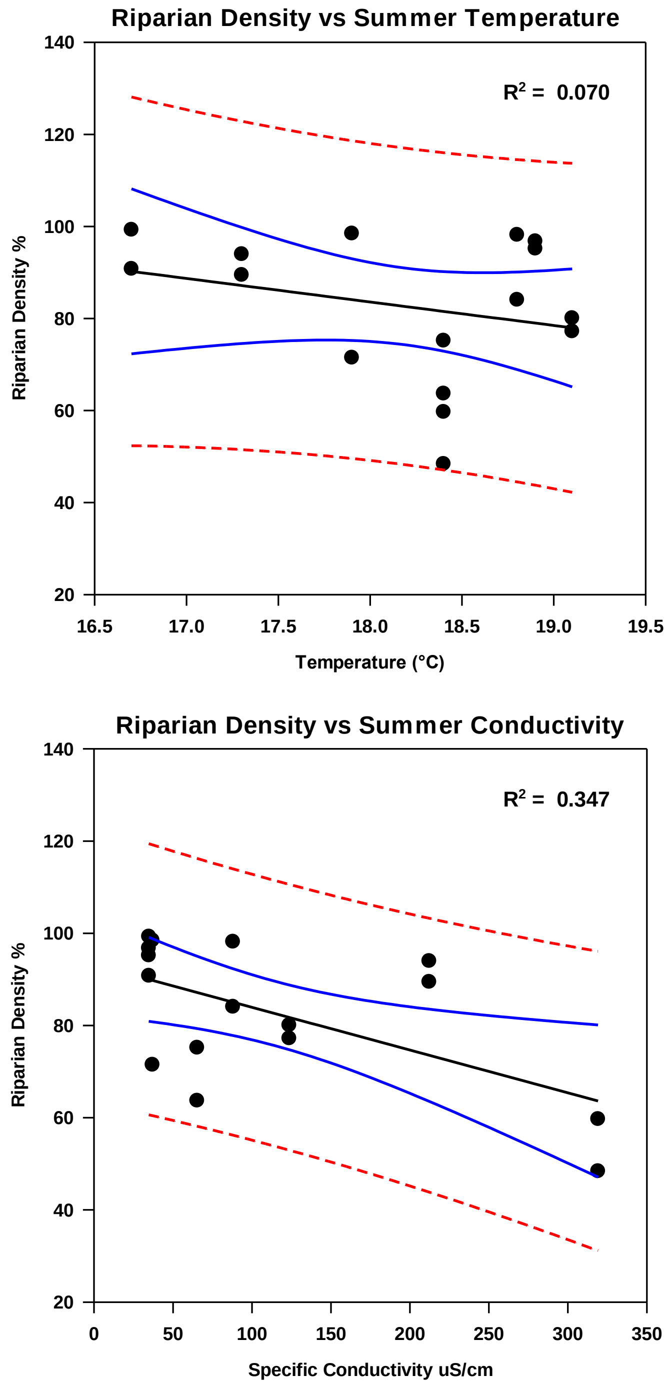


Fig. 21 Top: riparian density (%) compared to median summer temperature (°C) ($P = 0.321$); bottom: riparian density (%) compared to median specific conductivity (µS/cm) values ($P = 0.016$).

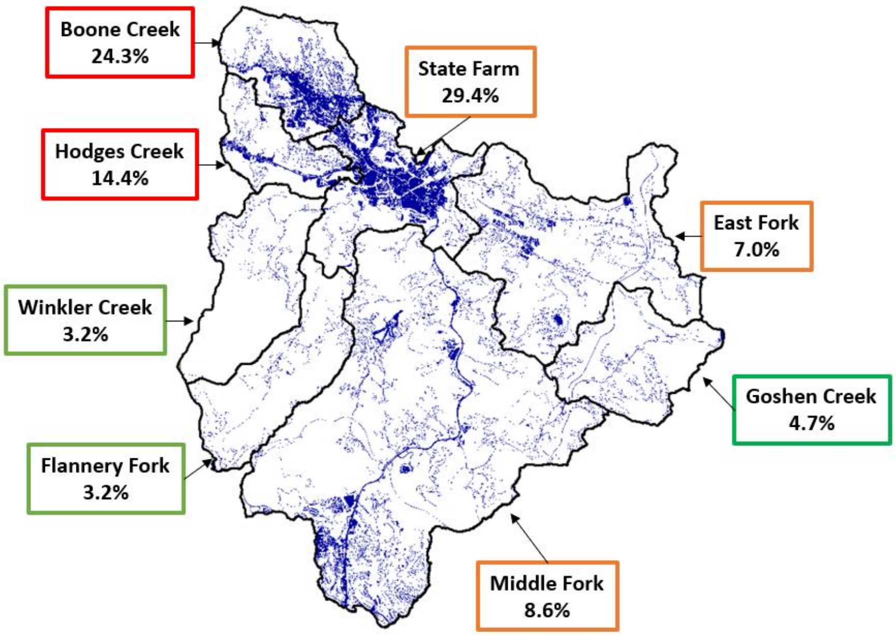
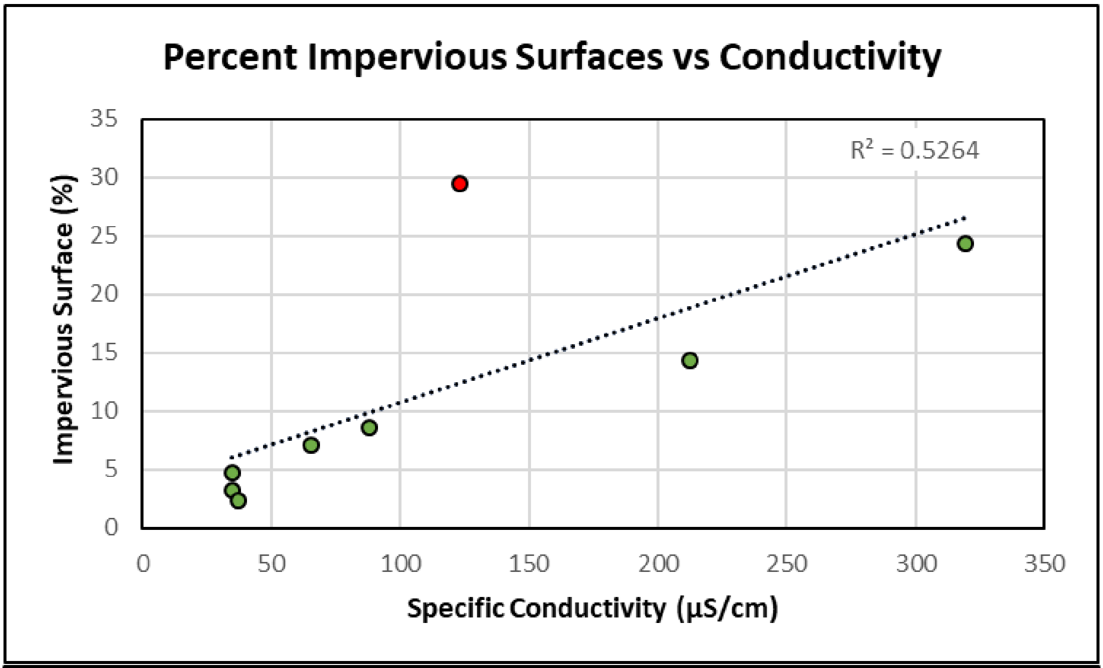


Fig. 22 Impervious surfaces land-cover map of the USFNR watershed and sub-basins. Black outlines = sub-basin boundaries; blue = impervious surfaces; white = non-impervious surfaces. Sub-basin names are color coded based on impact level (green = reference; orange = moderate; red = high).



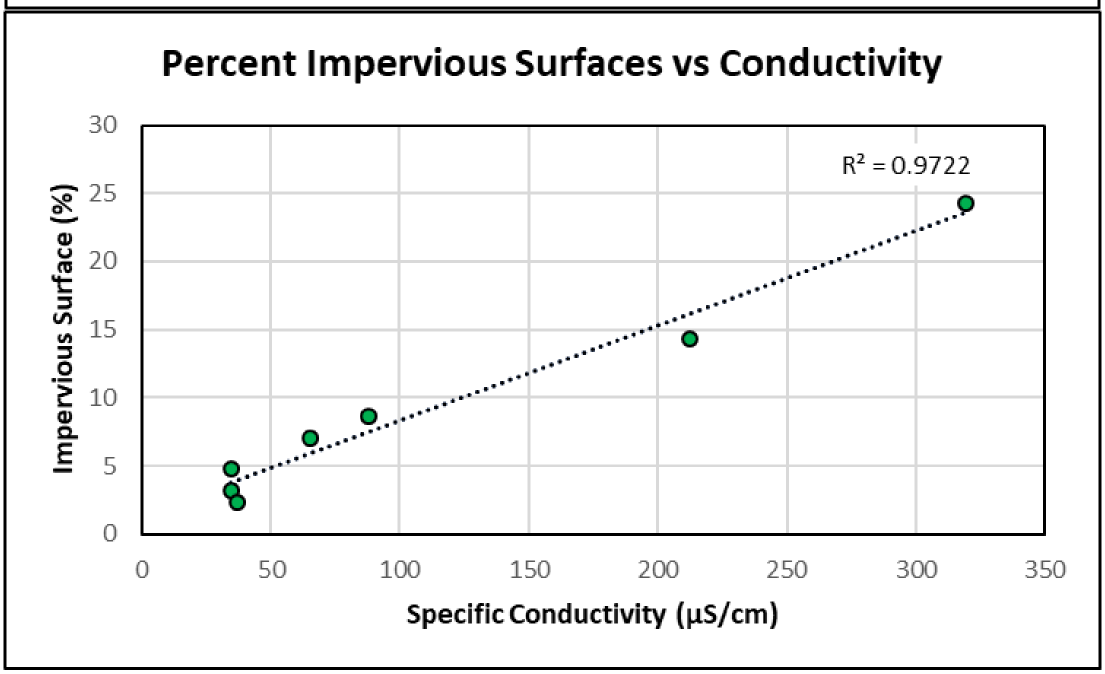


Fig. 23 Top: regression of the percentages of impervious surfaces on a sub-basin level compared to concentrations of specific conductivity (µS/cm); bottom: regression of the percentages of impervious surfaces on a sub-basin level compared to concentrations of specific conductivity (µS/cm) with outlier removed.

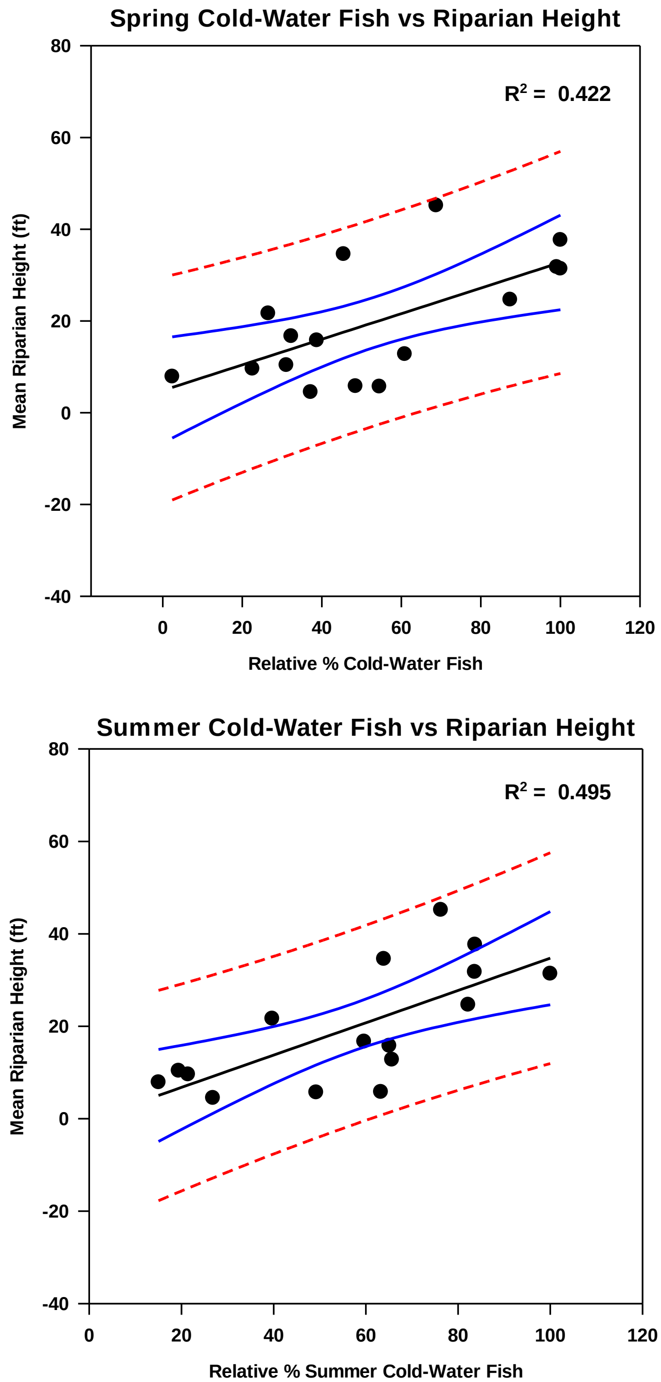
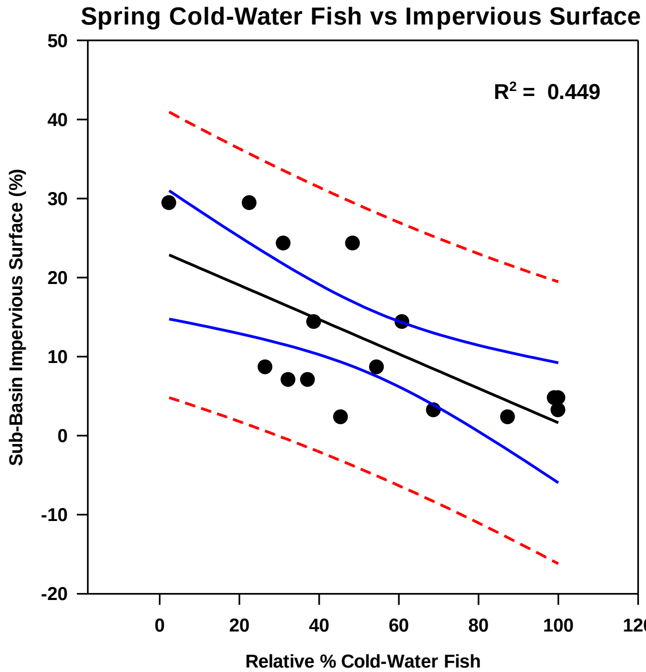


Fig. 24 Regression analysis comparing relative percentages of cold-water fish species in spring (top; P = 0.006) and summer (bottom; P = 0.002) to average riparian corridor heights.



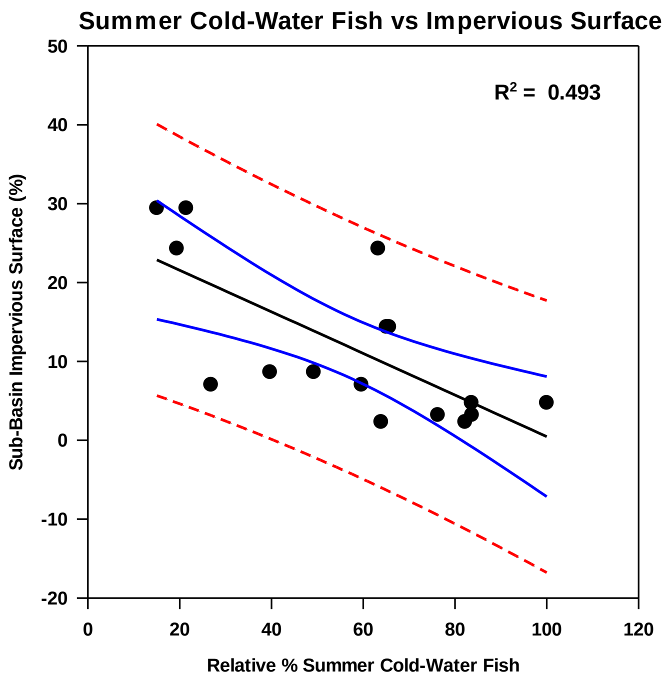


Fig. 25 Regression analysis comparing relative percentages of cold-water fish species in the spring (top; P = 0.004) and summer (bottom; P = 0.002) to sub-basin wide percentages of impervious surface.

Vita

Cristina Sanders was born in California and was raised in a small, rural town in Idaho where she quickly grew a passion for the outdoors and the protection of wildlife. She became especially fond of salmonids at a young age from watching the annual salmon runs in the river behind her parents' cabin. Cristina grew up surrounded by wilderness and enjoyed camping, hiking, hunting, and every other outdoor activity imaginable. In 2008, Cristina graduated from Heartland High School and joined the U.S. Army as a wheeled vehicle mechanic at Fort Bragg, NC, shortly after. She committed 4.5 years to active service, including a deployment to Afghanistan in 2010. Following deployment, she had her son Landon and completed her active duty contract with the U.S. Army and joined the North Carolina National Guard. Overall, she committed 11 years to the U.S. military before retiring as a sergeant in 2019.

In 2013, Cristina was working full-time and decided to pursue her passion for the environment by enrolling at the University of Phoenix for a B.S. degree in Environmental Sciences, which she completed in 2016. In 2017, she decided to continue her education by attending graduate school at Appalachian State University. Cristina started graduate school in the face of a steep learning curve since this was her first experience with a "brick and mortar" university. She applied her enthusiasm for salmonids by studying the environmental impacts on cold-water fish in her thesis work.

Cristina is continuing her journey working for the U.S. Fish and Wildlife Service as a Fish Biologist in Dexter, NM.